Carbohydrate Research, 214 (1991) 325–330
Elsevier Science Publishers B.V., Amsterdam

Cross-linked, degradable starch microspheres as carriers of paramagnetic contrast agents for magnetic resonance imaging: Synthesis, degradation, and relaxation properties

Pål Rongved,
Nycomed AS, R & D Division, P. O. Box 4220 Torshov, N-0401 Oslo 4 (Norway)

Bernt Lindberg,
Pharmacia AB, Department of Chemical Research, S-751 82, Uppsala (Sweden)

and Jo Klaveness*
Hafslund Nycomed AS, Corporate R & D, P.O. Box 5010 Majorstua, N-0301 Oslo 3 (Norway)

(Received April 2nd, 1990; accepted for publication, October 26th, 1990)

ABSTRACT

Biodegradable particles were produced by the cross-linking of starch with epichlorohydrin. Diethylenetriaminepenta-acetic acid (DTPA) was covalently linked to the particles by using DTPA bisanhydride. The small, gadolinium-labelled particles were 40–260% more efficient *in vitro* proton relaxation agents than the corresponding unbound chelate gadolinium–DTPA. The relaxation properties were dependent on the metal chelate, the particle size, the metal content, and the degree of substitution (d.s.). For the small gadolinium–DTPA particles, an increased d.s. decreased the rate of degradation by alpha-amylase.

INTRODUCTION

Magnetic resonance imaging (m.r.i.) is a new medical imaging modality which, for most areas of the human body, provides a better contrast resolution than other imaging techniques such as X-ray, ultrasound, and scintigraphy. Paramagnetic salts and chelates of lanthanides and transition metals are potential contrast agents in m.r.i., because of a characteristic effect on proton relaxation.

The spin–lattice relaxation rate of water protons can be enhanced by the addition of paramagnetic salts[1]. The first use of paramagnetic m.r.i. contrast agents was described by Lauterbur *et al.*[2], and various paramagnetic compounds have been evaluated in animals and humans. The first m.r.i. contrast agent to be marketed was the bismeglumine (1-deoxy-1-methylamino-D-glucitol) salt of gadolinium–diethylenetriaminepenta-acetic acid (Gd–DTPA), a well-tolerated chelate with broad potential clinical indications[3]. However, Gd–DTPA and other hydrophilic compounds of low molecular weight are "general contrast agents" with extracellular distribution and renal elimination. The main clinical indications for such compounds today are space-filling lesions in the central nervous system, and there is a need for more tissue-specific m.r.i. contrast agents. Lipophilic chelates have been investigated for the hepatobiliary sys-

* Author for correspondence.

0008-6215/91/$03.50

tem[4], porphyrins and monoclonal antibodies for tumor imaging[5,6], water-soluble macromolecules as potential blood-pool agents[7], and particles and liposomes for m.r.i. of liver and spleen[8]. Small particles administered intravenously will be cleared rapidly from the circulation by the reticuloendothelial systems with liver and spleen as the main target organs.

The aims of the study now reported were to synthesise and evaluate the biodegradability and *in vitro* efficacy of cross-linked starch microspheres loaded with paramagnetic chelates.

EXPERIMENTAL

General. — Spherical starch particles with various average diameters were prepared by cross-linking hydrolysed potato starch with epichlorohydrin by a modification of the bead polymerisation method[9,10]. The size of the particles was measured with a Coulter Counter.

Methyl sulfoxide was dried by storage over 4 Å molecular sieves. Half lives ($T_{1/2}$) were determined with 240 IU/L of alpha-amylase (Sigma) in phosphate buffer at pH 7.0 and 37°. Water regain (Wr) is defined as the weight of water (g) taken up by 1 g of dry particles.

The products were purified and isolated as follows. Each suspension was dialysed against aqueous 0.9% sodium chloride for 5 days. The external solution was exchanged 2–3 times every day until the T_1 was >3000 ms (see relaxation measurements). The suspension was then dialysed against distilled water and dried *in vacuo* at 50°. The metal content (%) in each product was determined by atomic absorption spectroscopy.

Iron(II)–DTPA–starch particles (**1**). — DTPA bisanhydride[11] (DTPA-A) (1.5 g) was added to a suspension of swollen starch microspheres (2.0 g, diam. 50 μm) in dry methyl sulfoxide (60 mL) at ambient temperature. The suspension was agitated at ambient temperature for 24 h, then cooled with an ice–water bath. Distilled water (100 mL) was added and the suspension was agitated at ambient temperature for 1 h. The particles were collected by centrifugation, and washed 6 times by alternate suspension in distilled water and centrifugation. Finally, the pH of a suspension in distilled water (50 mL) was adjusted to 6.2, and a solution of $FeCl_2 \cdot 4H_2O$ (0.92 g) in distilled water (10 mL) was added with agitation. The pH was adjusted to 5.1, the suspension was agitated for 2 h, and the particles were isolated by centrifugation, washed with distilled water, purified, and isolated as dark-yellow particles (0.9 g) (Fe, 5.6%).

Copper(II)–DTPA–starch particles (**2**). — DTPA was bound to starch microspheres (2.0 g) as described above. The pH of a suspension of the particles in distilled water (50 mL) was adjusted to 6.1, a solution of $CuSO_4 \cdot 5H_2O$ (1.15 g) in distilled water (10 mL) was added with agitation, the pH was adjusted to 5.2, and the suspension was agitated for 1 h. The particles were purified and isolated as blue–green particles (1.2 g) (Cu, 8.1%).

Gadolinium–DTPA–starch particles (**3**). — DTPA was bound to starch particles (0.3 g) as described above. The pH of a suspension of the particles in distilled water (30 mL) was adjusted to 5.8 and a solution of $GdCl_3 \cdot 6H_2O$ (0.2 g) in distilled water (20 mL) was added with agitation. The pH was adjusted to 5.8, the suspension was agitated for 1 h, and the particles were purified and isolated as white particles (0.25 g) (Gd, 1.5%).

Erbium(III)–DTPA–starch particles (**4**). — DTPA was bound to starch particles (2.0 g) as described above. The pH of a suspension of the particles in distilled water (50 mL) was adjusted to 6.2, a solution of $ErCl_3$ (1.77 g containing 40% of water) in distilled water (10 mL) was added with agitation, the pH was adjusted to 5.1, and the suspension was agitated for 30 min. The particles were purified and isolated as white particles (1.77 g) (Er, 8.5%).

Chromium(III)–DTPA–starch particles (**5**). — DTPA was bound to starch particles (2.0 g, diam. 1.5 μm) as described above. The pH of a suspension of the particles in distilled water (50 mL) was adjusted to 6.1, and a solution of $CrCl_3 \cdot 6H_2O$ (1.23 g) in distilled water (10 mL) was added. The pH was adjusted to 5.0 and the suspension was agitated for 35 min. The particles were purified and isolated as violet particles (1.23 g) (Cr, 4.0%).

Manganese(II)–DTPA–starch particles (**6**). — DTPA was bound to starch particles (2.0 g, diam. 1.5 μm) as described above. The pH of a suspension of the particles in distilled water (50 mL) was adjusted to 6.2 and a solution of $MnCl_2 \cdot 4H_2O$ (0.91 g) in distilled water (10 mL) was added. The pH was adjusted to 5.2 and the suspension was agitated for 40 min. The particles were purified and isolated as white particles (0.91 g) (Mn, 5.9%).

Iron(III)–DTPA–starch particles (**7**). — DTPA was bound to starch particles (2.0 g, diam. 1.5 μm) as described above. The pH of a suspension of the particles in distilled water (50 mL) was adjusted to 6.3 and a solution of $FeCl_3 \cdot 6H_2O$ (1.25 g) in distilled water (10 mL) was added. The pH was adjusted to 5.1 and the suspension was agitated for 1 h. The particles were purified and isolated as dark-yellow particles (1.25 g) (Fe, 7.8%).

Gadolinium(III)–DTPA–starch particles (**8**). — DTPA was bound to starch particles (2.0 g, diam. 1.5 μm) as described above. The pH of a suspension of the particles in distilled water (50 mL) was adjusted to 6.1 and a solution of $GdCl_3 \cdot 6H_2O$ (1.72 g) in distilled water (10 mL) was added with agitation. The pH was adjusted to 5.2 and the suspension was agitated for 50 min. The particles were purified and isolated as white particles (1.72 g) (Gd, 12.2%).

Gadolinium(III)–DTPA–starch particles (**9–12**). — Different amounts of Gd–DTPA were bound to starch particles (diam. 1.5 μm) as described for **8**. See Table I for experimental details.

Relaxation measurements. — All measurements were performed in glycerol–water (1:2.13) at 0.24 T and 37° on a Radx n.m.r. spectrometer (Radx Proton Spin Analyzer), using an inversion recovery sequence. The spin–lattice relaxivities (r_1) are listed in Table II.

TABLE I

Preparation of gadolinium–DTPA–starch microspheres[a] **9–12**

Product	*DTPA–A (mmol)*	*$GdCl_3$ (mmol)*	*Yield (g)*	*Gd (%)*	*Wr*
9	1.0	0.9	1.2	8.2	12.0
10	0.5	0.45	1.1	5.2	14.9
11	0.25	0.225	1.0	3.2	16.8
12	0.1	0.09	1.0	1.1	13.0

[a] From 1.0 g of starch particles, which corresponds to ~5.5 mmol of glucose.

TABLE II

T_1 relaxivity of paramagnetically labelled DTPA–starch microspheres (**1–12**) in glycerol–water

Product	*Metal ion (M)*	*Diameter (μm)*	*M (%)*	*r_1^a (s^{-1} mmol^{-1} L)*
1	Fe(II)	60	5.6	0.22
2	Cu(II)	50	8.1	0.12
3	Gd(III)	50	1.5	3.0
4	Er(III)	70	8.5	0.11
5	Cr(III)	1.2	4.0	0.91
6	Mn(II)	1.4	5.9	2.95
7	Fe(III)	1.9	7.8	0.53
8	Gd(III)	1.3	12.2	6.4
9	Gd(III)	1.3	8.2	6.9
10	Gd(III)	1.3	5.2	9.1
11	Gd(III)	1.3	3.2	11.1
12	Gd(III)	1.3	1.1	15.9

[a] All relaxivity values are denoted per metal concentration.

DISCUSSION

Degradable starch microspheres can be prepared with physiochemical properties within well defined limits[12,13]. These microspheres are slightly deformable and have a sharp distribution of sizes. In the microspheres used for preparation of **1–4**, 65–70% of the glucose residues remained unsubstituted after the cross-linking process[12]. The particles swelled markedly in water and possessed a typical gel-like character in an aqueous medium. Water-soluble carbohydrates have been evaluated as potential carriers of paramagnetic m.r.i. contrast agents[14].

Data on the composition and relaxivity of derivatives **1–12** are summarised in Table II. The products had particle-size distributions in the range 1.2–70 μm. Table I contains details for the preparation of gadolinium–DTPA particles (**9–12**). This method of paramagnetic labelling of the particulate material was highly reproducibile and the metal content in the products was linearly dependent on the quantities of the reagents used.

The measurements of relaxivity were performed at 10 MHz in glycerol–water (1:2.13), which has relaxation properties close to those of body fluids. The significant difference in relaxivity of, for example, Gd–DTPA and Fe(III)–DTPA in this medium and distilled water was due to the increased viscosity.

The spin–lattice relaxivities (r_1) for **8–12** were 40–260% higher than that for Gd–DTPA, even though the paramagnetic units were attached to a solid or semisolid material. Runge *et al.*[15] have suggested gadolinium oxalate particles as potential m.r.i. contrast agents. The *in vitro* relaxivity of gadolinium oxalate in water with respect to mM gadolinium was 100-fold less than, for example, **12** in our study[22]. The gadolinium oxalate particles are crystal-like, apparently with a low degree of solvation of the paramagnetic centres, and with a relatively large part of the gadolinium atoms inaccessible to the bulk water. Since the starch microspheres swell in water, the diffusion into the matrix results in efficient relaxation of the bulk water. There is an increase in r_1 with decreasing gadolinium content from $> 12\%$ for **8** to $\sim 1\%$ for **12**. There will be an increase in the number of cross-links near to each paramagnetic centre; this may result in some decrease in the tumbling rate of the surrounding water, which in turn will affect the relaxation rate.

The Gd–DTPA derivative **3** has a gadolinium content intermediate of those of **11** and **12**, but only 20–25% of their relaxivity. Small particles have a larger surface area and, apparently, this may affect the relaxivity.

The starch-particle derivatives of the DTPA chelates of the paramagnetic metals **1–7** have r_1 values of the same order of magnitude as those of the DTPA chelates (Table III).

Enzymic degradation of the paramagnetic Gd-labelled starch microspheres **8–12** is summarised in Table IV. Lindberg *et al.*[12] described the mechanism of the degradation of starch particles by alpha-amylase in connection with studies of the arrest of blood flow. The half life ($T_{1/2}$) was defined as the time needed for 50% of the mass of the particles to dissolve; $T_{1/2}$ is proportional to the average diameter and the degree of cross-linking of the particles. The results in Table IV also show that $T_{1/2}$ is proportional

TABLE III

T_1 relaxivity of DTPA chelates

Metal ion	r_1 ($s^{-1}.mmol^{-1}.L$)	*Frequency (MHz)*	*Temp. (°)*	*Medium*[a]	*Ref.*
Fe(III)	1.3	10	37	A	22
Fe(III)	0.72	20	37	B	20
Cu(II)	0.12	60	20	B	21
Er(III)	<0.10	10	37	B	22
Cr(III)	0.20	10	37	B	22
Mn(II)	1.3	20	35	B	23
Gd(III)	4.1	20	35	B	23
Gd(III)	6.0	10	37	A	22

[a] A, Glycerol–water (1:2.13); B, distilled water.

TABLE IV

Degradation of gadolinium–DTPA-labelled starch microspheres **8**–**12** by alpha-amylase

Substrate	*Gd* (%)	$T_{1/2}$ (*h*)
8	12.2	> 24
9	8.2	8
10	5.2	5.5
11	3.2	1.5
12	1.1	0.67

to the degree of substitution. Thus, the substituents may block the action of the enzyme or the reaction of the DTPA bisanhydride may increase the degree of cross-linking and affect $T_{1/2}$. In addition, trace amounts of gadolinium ions might, like lanthanium ions, interact with calcium ions in the alpha-amylase and modify its activity[16]. Experiments are in progress to answer these questions.

ACKNOWLEDGMENTS

We thank Mr. Søren Svensson (Pharmacia AB) for excellent technical assistance in the preparation of microspheres.

REFERENCES

1 F. Bloch, W. W. Hansen, and M. Packard, *Phys. Rev.*, 70 (1946) 474–485.
2 P. C. Lauterbur, M. H. Mendonca-Dias, and A. M. Rudin, in P. L. Dutton, J. S. Leigh, and A. Scarpa, (Eds.), *Frontiers of Biological Energetics*, Academic Press, New York, 1978, pp. 752–759.
3 H. P. Niendorf and J. Haustein, in P. A. Rinck (Ed.), *Contrast and Contrast Agents in Magnetic Resonance Imaging*, The European Workshop on Magnetic Resonance in Medicine, Mons, 1989, pp. 21–32.
4 R. B. Lauffer, in V. M. Runge (Ed.), *Enhanced Magnetic Resonance Imaging*, Mosby, St. Louis, 1989, pp. 129–135.
5 R. C. Lyon, P. J. Faustino, J. S. Cohen, and A. Katz, *Magn. Reson. Med.*, 4 (1987) 24–33.
6 E. C. Unger, W. G. Totty, D. M. Neufeld, F. L. Otsuka, W. A. Murphy, M. S. Welch, J. M. Connett, and G. W. Philpott, *Invest. Radiol.*, 20 (1985) 693–700.
7 U. Schmiedl, M. E. Moseley, R. Sievers, M. D. Ogan, W. M. Chew, H. Engeseth, W. E. Finkbeiner, M. J. Lipton, and R. C. Brasch, *Invest. Radiol.*, 22 (1986) 713–721.
8 J. Klaveness, in ref. 4, pp. 117–128.
9 U. S. Rothman and B. J. Lindberg, Ger. Pat. 2 524 278 (1975); *Chem. Abstr.*, 85 (1976) 25 381.
10 B. G. A. Ingelman and M. Ceska, Fr. Pat. 2 000 245 (1969); *Chem. Abstr.*, 73 (1970) 84 192.
11 W. C. Eckelmann, S. M. Karesh, and R. C. Reba, *J. Pharm. Sci.*, 64 (1975), 704–706.
12 B. Lindberg, K. Lote, and H. Teder, in S. S. Davis, L. Illum, J. G. McVie, and E. Tomlinson (Eds.), *Microspheres and Drug Therapy*, Elsevier, Amsterdam, 1984, pp. 153–188.
13 R. F. Tuma, in ref. 12, pp. 189–203.
14 P. Rongved and J. Klaveness, *Carbohydr. Res.*, 214 (1991) 315–323.
15 V. M. Runge, R. G. Stewart, J. A. Clanton, M. M. Jones, C. M. Lukehart, C. L. Partain, and A. E. James, Jr., *Radiology*, 147 (1983) 789–791.
16 C. H. Evans, *Trends Biochem. Sci.*, (1983) 445–449.
20 S. H. Koenig and R. D. Brown III, in H. Y. Kressel (Ed.), *Magnetic Resonance Annual*, Raven Press, New York, 1987, pp. 263–286.
21 J. Oakes and C. G. van Kralingen *J. Chem. Soc., Dalton Trans.*, (1984) 1133–1137.
22 P. Rongved and J. Klaveness, unpublished results.
23 S. H. Koenig, C. Baglin, R. D. Brown III, and C. F. Brewes, *Magn. Reson. Med.*, 1 (1984) 496–501.