Peptides Form Stereoselective Complexes with Chiral Polymers

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Stereoselective interactions between pairs of opposite enantiomeric polymers have been sporadically reported since the early 1960s.[1−4] Also, pairs of D- and L-poly(lactic acid), either as homopolymers or in block copolymers, were reported to form a stereocomplex.[5−9] Striking changes in physical and chemical behavior were observed for this stereocomplex, compared to its parent compounds, like its insolubility in common solvents, a raise in melt transition temperature of 50 deg to 230 °C, and changes in solid-state NMR spectra.[10,11]

Never has any attempt been made to form stereoselective complexes of nonidentical pairs of polymers with opposite chirality. We report here for the first time on the complex formation between naturally L-oriented polypeptides and poly(D-lactic acid).

When comparing peptides and proteins to poly(lactic acid) (PLA), a close similarity was found in torsion angles and bond lengths.[12] Homo-stereocomplexes of D-PLA and L-PLA were reported to consist of intertwined 3_1 helices, formed from 10_3 helices of the corresponding enantiomers.[7] It is thus suggested that, similarly, the hetero-stereoselective L-peptide/D-PLA complexes consist of intertwined helices, forming a physical complex.

L-Configured leuprolide—a luteinizing hormone-releasing hormone (LHRH) nonapeptide analogue—and vapreotide—a cyclic octapeptide somatostatin analogue—were used as representative peptides for hetero-stereocomplex formation with D-PLA. Complexes were readily obtained as precipitate from an acetonitrile/water solution after stirring at 60 °C for 3 days. Wide-angle X-ray scanning (WAXS) and solid- or liquid-state NMR and FT-IR spectra showed no alteration from the starting polymers, indicating no changes in chemical bonds both in the poly(lactic acid) and in the peptide. Over 95% of the peptide was complexed, whereas less than 5% of free peptide was detected in solution by HPLC. Under identical reaction conditions neither L-PLA nor racemic DL-PLA formed any complex with polypeptides.

In the formation of the stereoselective complex, a precipitate of highly porous uniform spheres of 1−3 μm in diameter was obtained as shown by scanning electron microscopy (SEM) (Figure 1A). Compared to regular smooth microspheres, prepared by known encapsulation methods,[13,14] these fibrous spheres displayed a far larger surface area.

A clear indication of the stereoselective nature of the complex formation was obtained by small-angle X-ray scattering (SAXS) analysis. Upon mixing solutions of D-PLA and leuprolide, a rapid increase in scattering intensity was recorded within a few minutes, showing the initial formation of small particles. Under identical conditions, neither L-PLA nor racemic DL-PLA showed any interaction with leuprolide (Figure 2).

On the basis of indirect Fourier transformation of the scattering intensity data, particle form and sizes were

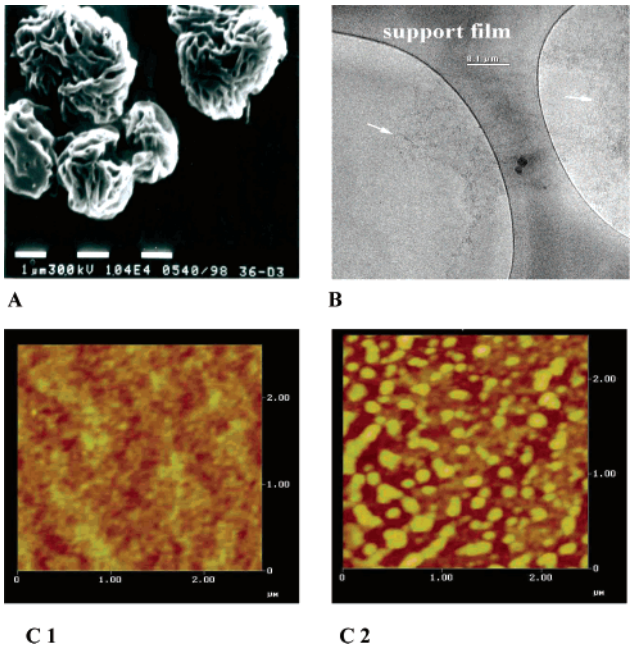

Figure 1. (A, B) Scanning electron microscopy: (A) gold-coated D-PLA(M_w 120 kDa)/vaprotide (2% w/w) stereoselective complex, precipitated from an acetonitrile solution; (B) Cryo-TEM of D-PLA(M_w 100 kDa)/leuprolide (25% w/w) stereoselective complexes in DMF, trapped in the vitrified solvent film in the holes of the support film 2 min after mixing polymer and peptide solution at 60 °C. (C1 and C2**)** Atomic force microscopy−tapping mode: (C1**)** 0.1% D-PLA(M_w 100 kDa) in acetonitrile, cast on freshly cleaved mica; (C2) 0.025% leuprolide solution in water/acetonitrile at 60 °C, cast on top of previous D-PLA layer, left at 60 °C for drying.

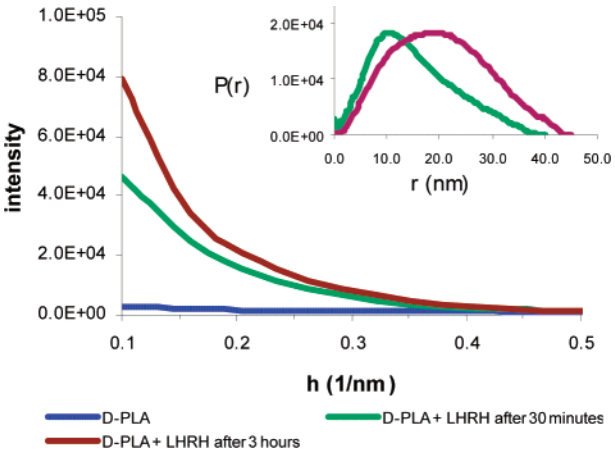

Figure 2. SAXS of D-PLA(M_w 100 kDa)/leuprolide stereoselective complex. Acetonitrile/water (9:1) solution of D-PLA 1% w/v and leuprolide 0.25% w/v in sealed capillary at 60 °C. $h=4\pi\sin\theta/\lambda$ where 2θ is the scattering angle and λ the wavelength. Inset: the distance distribution function P(r) obtained by the Fourier transform procedure.[15]

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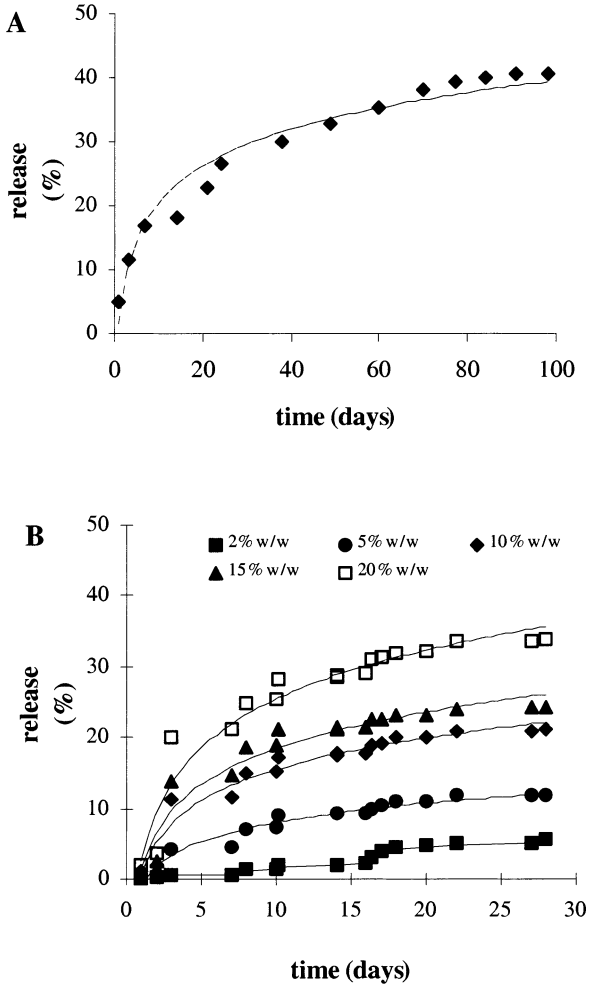

Figure 3. Controlled release from stereoselective complexes: (A) leuprolide release from complex with D-PLA 3.5 kDa, 5% loading w/w polymer; (B) vapreotide release from complex with D-PLA 120 kDa in various concentrations w/w. Complexes were prepared by reacting in acetonitrile at 60 °C for 3 days.

calculated.[15] D-PLA (molecular weight of 100 kDa) initially formed elongated particles (30 × 40 nm) with leuprolide, changing after a few hours to spherical particles (50 nm). D-PLA of a smaller molecular weight (10 kDa), however, immediately formed spherical particles of 50 nm in the complexation with leuprolide. These particles, initially formed within 2 min after mixing D-PLA with leuprolide, were visualized by cryogenic transmission electron microscopy (Cryo-TEM), using DMF as reaction medium. The white arrows in the Cryo-TEM scans, shown in Figure 1B, point to the entrapped nanoparticles in the vitrified DMF film, formed in the holes of the support film. The specimen was quenched from 50 °C, using a controlled environment vitrification system (CEVS).

At ambient temperature and atmospheric pressure stereoselective complex particles were imaged by atomic force microscopy−tapping mode. Disklike particles were observed when casting a leuprolide solution at 60 °C on top of D-PLA, deposited on freshly cleaved mica. The previously observed fibrous network of D-PLA almost totally disappeared (Figure 1, C1 and C2). The diameter of the disklike particles was found between 100 and 200 nm and the average height 20 nm.

The stereoselective complexes provide a novel approach in controlled delivery of peptides and proteins, relying primarily on the detachment of the complex, which depends on the stereo-interactions between the peptide and D-PLA at a molecular level. On the basis of the high porosity of the formed particles and the relatively long release period of the various peptides, we suggest that the release is not diffusion dependent, found in most common peptide and protein release devices, composed of biodegradable polymers,[14,16] but depends on detachment of the complex. When suspended in 0.1 M phosphate buffer solution, pH 7.4, at 37 °C at a constant shaking of 100 rpm, the complex particles released their peptide contents over a period of 1−3 months in a first-order pattern (Figure 3). At specific time intervals the buffer was removed, and the amount of released peptide was measured using HPLC.

In summary, we have discovered reversible stereoselective complexes between L-peptides and enantioselective D-polyesters. The spontaneously formed complexes obtained as uniform microparticles of 2−5 μm in size showed controlled release of the interlocked peptides. The stereoselective complexation occurred only with D-PLA, not with L-PLA or racemic DL-PLA as shown by SAXS studies.

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References and Notes

(1) Liquori, A. M.; Anzuino, G.; Coiro, V. M.; D'Alagni, M.; de Santis, P.; Savino, M. *Nature (London)* **1965**, *206*, 358−362.
(2) Baba, Y.; Kagemoto, A. *Macromolecules* **1977**, *10*, 458−460.
(3) Grenier, D.; Prud'homme, R. E. *J. Polym. Sci., Polym. Phys.* **1984**, *22*, 577−587.
(4) Iribarren, I.; Aleman, C.; Regano, C.; delIarduya, A. M.; Bou, J. J.; MunozGuerra, S. *Macromolecules* **1996**, *29*, 8413−8424.
(5) Ikada, Y. J., Kh.; Tsuji, H.; Hyon, S.-H. *Macromol. Macromol.* **1987**, *20*, 904−906.
(6) Stevels, W. M.; Ankone, M. J. K.; Dijkstra, P. J.; Feijen, J. *Macromol. Chem. Phys.* **1995**, *196*, 3687−3694.
(7) Brizzolara, D.; Cantow, H. J.; Diederichs, K.; Keller, E.; Domb, A. J. *Macromolecules* **1996**, *29*, 191−197.
(8) de Jong, S. J.; De Smedt, S. C.; Wahls, M. W. C.; Demeester, J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Macromolecules* **2000**, *33*, 3680−3686.
(9) Pensec, S.; Leroy, M.; Akkouche, H.; Spassky, N. *Polym. Bull. (Berlin)* **2000**, *45*, 373−380.
(10) Tsuji, H.; Hyon, S. H.; Ikada, Y. *Macromolecules* **1992**, *25*, 2940−2946.
(11) Tsuji, H.; Horii, F.; Nakagawa, M.; Ikada, Y.; Odani, H.; Kitamaru, R. *Macromolecules* **1992**, *25*, 4114−4118.
(12) De Santis, P.; Kovacs, A. J. *Biopolymers* **1968**, *6*, 299−306.
(13) Perrin, D. E.; English, J. P. In *Polyglycolide and Polylactide*, 1st ed.; Domb, A. J., Kost, J., Wiseman, D. M., Eds.; Harwood Academic Publishers: Amsterdam, 1997; Vol. 7, pp 3−28.
(14) Thies, C. *A Survey of Microencapsulation Processes*; Benita, S., Ed.; Marcel Dekker: New York, 1996; Vol. 73, pp 1−19.
(15) Glatter, O.; Kratky, O. *Small Angle Scattering*; Academic Press: London, 1982.
(16) Bartus, R. T.; Tracy, M. A.; Emerich, D. F.; Zale, S. E. *Science* **1998**, *281*, 1161−1162.

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