$^5J_{HCCOCH}$ couplings may assume larger values[6] and, accordingly, may result in the appearance of respective cross peaks rendering thereby the localization of the glycosidic linkages less straightforward. Ambiguities as to the site of glycosylation can presumably be removed either by the determination of the relative signs of interresidual long-range couplings ($^4J_{HOCH}$ and $^5J_{HCOCCH}$ are supposed respectively to be of negative and positive signs[6,10]) or by proton–carbon chemical-shift correlations. It may be noted that in our sequencing experiments[11] with other di- and trisaccharides including Bzl β-D-Xylp-(1→4)-α-L-Rhap, Bzl β-L-Xylp-(1-4)-α-L-Rhap, Me α-L-Rhap-(1→4)-α-L-Rhap-(1→2)-α-L-Rhap, and Me α-L-Rhap-(1→4)-α-L-Rhap-(1→3)-α-L-Rhap we failed to detect cross peaks due to five-bond interresidual interactions whereas the observation of four-bound interunit couplings caused no particular difficulties.

It can be expected that the experimental approach outlined above may be successfully employed for the sequencing of oligosaccharides containing a larger number of sugar units, provided the spectral dispersion is increased accordingly. It should, however, be emphasized that the detectability of magnetization transfer in a COSY experiment is governed primarily by the actual values of pertinent proton T_2 relaxation times.[7] Thus, in low-viscosity solutions, internal flexibility of the oligomer may be necessary to take full advantage of the higher magnetic fields.

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Registry No. 1, 87985-51-1.

Supplementary Material Available: ^{1}H NMR parameters of **1** and spectrum simulation compared with the experimental spectrum is presented (3 pages). Ordering information is given on any current masthead page.

(10) Relative signs of intraresidual long-range couplings were determined in a separate COSY-45 experiment performed with the methyl glycoside of the repeating unit, Me α-L-Rhap. It was found that $^4J_{HCOCH} < 0$ while $^5J_{HCOCCH} > 0$. (See also supplementary material.)

(11) Batta, Gy.; Liptâk, A. European Symposium on Carbohydrates and Glycoconjugates, 2nd, Budapest, August 9-12, 1983, Abstract E-12.

pH-Dependent Ion Transport across Polymer Membrane. pH-Induced Reversible Conformational Change of Transmembrane Poly(L-aspartic acid) Domain in Polymer Membrane

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Biological membranes consist of a continuous nonpolar hydrocarbon matrix from the phospholipid bilayer substantially impermeable to most polar substances and protein molecules capable of reversibly binding specific substrates and of transporting them across the membrane even against a gradient of concentration. The protein molecules may undergo reversible conformational changes to create a "hole" or a "channel" in the membrane for the specific substrate transported.[1]

In order to mimic the function of biomembrane, i.e., specific, facilitated, and/or active transports, numerous studies on model systems have been reported, most of which have examined the transport through fluid membranes or lipid-bilayer membranes by the aid of naturally occurring and synthetic ionophores.[2]

(1) Penrose, W. R.; Zand, R.; Oxender, D. L. *J. Biol. Chem.* **1970**, *245*, 1432.

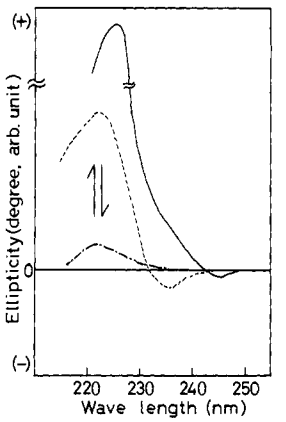

Figure 1. Circular dichroism spectra of the graft copolymer film measured by using a JASCO J-500A spectropolarimeter: (—) before hydrolysis; (---) after hydrolysis, at pH 3; (-·-) after hydrolysis, at pH 9.

However, such mobile carrier-mediated transports appear not to be very common in natural systems, and neither liquid membranes nor lipid-bilayer membranes are satisfactory as model systems and for possible practical applications because of their low stability. As for carrier immobilized on stable polymer membranes, various kinds of polymers have been examined.[3-6] However, they may not be regarded as a biomembrane model since the morphology of these membranes is different from that of biomembrane, where the most important feature is its mosaic structure.[7]

Our primary purpose is to compose the biomembrane model having transmembrane permeating pathway from synthetic macromolecules. In the present paper will be described the first example of the pH-induced reversible conformational change in the transmembrane polypeptide domain of the synthetic membrane prepared from butyl methacrylate (backbone)–L-aspartic acid (branch) graft copolymer.

The design of membrane with microdomains of poly(amino acid) in the matrix of vinyl polymers is based on the synthesis of vinyl polymers with poly(amino acid) branches,[8,9] some of which were found to have microdomains of poly(amino acid) similar to biomembrane.[8] In the present study, butyl methacrylate (backbone)–β-benzyl L-aspartate (branch) comb-type graft copolymer was synthesized by a similar procedure to that reported previously[9] and hydrolyzed in the form of membrane to prepare the membrane of butyl methacrylate–L-aspartic acid graft copolymer.

Figure 1 shows the circular dichroism (CD) spectra of the film cast on a quartz plate (5 mm thick) from the chloroform solution of the graft copolymer[10] before and after hydrolysis. Before

(2) (a) Pressman, B. C. "Inorganic Biochemistry"; Eichhorn, G. L., Ed.; American Elsevier: New York, 1973; Vol. 1, pp 203-226. (b) Behr, J. P.; Lehn, J. M. *J. Am. Chem. Soc.* **1973**, *95*, 6108. (c) Shinbo, T.; Kurihara, K.; Kobatake, Y.; Kamo, N. *Nature (London)* **1977**, *270*, 277. (d) Maruyama, K.; Tsukube, H.; Araki, T. *J. Am. Chem. Soc.* **1980**, *102*, 3246. (e) Lamb, J. D.; Christensen, J. J.; Izatt, S. R.; Bedke, K.; Astin, M. S.; Izatt, R. M. *Ibid.* **1980**, *102*, 3399. (f) Hiratani, K.; Aiba, S.; Nakagawa, T. *Chem. Lett.* **1980**, 477. (g) Hiratani, K. *Ibid.* **1981**, 21. (h) Läuger, P. *J. Membrane Biol.* **1980**, *57*, 163.

(3) Uragami, T.; Nakamura, R.; Sugihara, M. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 141.

(4) Fyles, T. M.; McGavin, C. A.; Thompson, D. E. *J. Chem. Soc., Chem. Commun.* **1982**, 924.

(5) (a) Shimidzu, T.; Yoshikawa, M.; Hasegawa, M.; Chiba, H. *Kobunshi Ronbunshu* **1977**, *34*, 753. (b) Shimidzu, T.; Yoshikawa, M.; Kawakatsu, K. *Polym. J.* **1980**, *12*, 363. (c) Shimidzu, T.; Yoshikawa, M.; Hasegawa, M.; Kawakatsu, K. *Macromolecules* **1981**, *14*, 170.

(6) (a) Ogata, N.; Sanui, K.; Fujimura, H. *J. Polym. Sci., Polym. Lett. Ed.* **1979**, *17*, 753; (b) *J. Appl. Polym. Sci.* **1980**, *25*, 1419; (c) *Ibid.* **1981**, *26*, 4149.

(7) Nicolson, G. L. *Biochim. Biophys. Acta* **1976**, *457*, 57.

(8) Maeda, M.; Inoue, S. *Makromol. Chem., Rapid Commun.* **1981**, *2*, 537.

(9) Kimura, M.; Egashira, T.; Nishimura, T.; Maeda, M.; Inoue, S. *Makromol. Chem.* **1982**, *183*, 1393.

(10) The graft copolymer used in the CD measurement was that having the degree of branching of 2 mol % and the number-average degree of the polymerization ($\overline{DP_n}$) of the branch of 17. Molecular weight of the trunk was estimated as ca. 10 000 by gel permeation chromatography.

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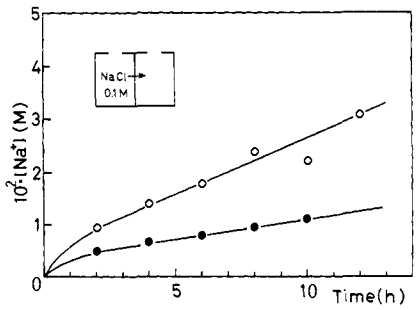

Figure 2. Changes in the concentration of Na^+ ion on the right side of the hydrolyzed membrane at 31.0 °C. Initial concentration of Na^+ ion on the left side, 0.1 M. (○) At pH 7; (●) at pH 3. A diaphragm cell that consisted of two detachable parts with the volume of 50 mL was used. Concentration of Na^+ ion transported was measured by atomic absorption photometry.

hydrolysis CD spectrum showed a positive curve centered at 226 nm, which represents that poly(β-benzyl L-aspartate) chains (branch) in the membrane take mainly left-handed α-helix, in a similar manner to homopolymer of β-benzyl L-aspartate in the solid state.[11]

When the hydrolyzed film[12] was fitted in a quartz cell (path length, 10 mm) filled with an aqueous solution of HCl (pH 3), CD spectrum still exhibited a positive curve centered at 222 nm, but of lower intensity than that of the film before hydrolysis. Relative maximum value of ellipticity at pH 3 of hydrolyzed film was 20% of that before hydrolysis. The positive peak at 222 nm indicates the formation of left-handed α-helix in contrast with the fact that poly(L-aspartic acid) forms right-handed α-helix in acidic aqueous solution like other polypeptides from L-amino acid.[13]

When the hydrolyzed film was immersed in an aqueous solution of NaOH (pH 9), a pronounced decrease in the intensity of the CD spectrum (from 20% to 3%, as the relative maximum value to that before hydrolysis) was observed, indicating the transformation from ordered to random coil form of the poly(amino acid) chain in the film. This behavior is fully reversible; upon lowering the pH (from 9 to 3) the same CD curve as the starting reappeared immediately.

A preliminary experiment of Na^+ ion transport across the hydrolyzed membrane[14] was carried out. As shown in Figure 2, Na^+ ion was found to permeate through the hydrolyzed membrane prepared from the graft copolymer. Transfer of water due to the osmotic pressure was also observed. On the other hand, Na^+ ion did not permeate through the membrane from the homopolymer of butyl methacrylate that was subjected to the same hydrolyzing condition. The graft copolymer that was not treated with the hydrolyzing solution was also impermeable for Na^+ ion. From these results, it is considered that continuous phases of poly(L-aspartic acid) domain are formed in the membrane and function as permeating pathways, or "transmembrane channels", for Na^+ ion. Under acidic condition (pH 3), the transport rate of Na^+ ion decreased significantly compared with that under neutral condition (pH 7), which is considered to be ascribed, at least partly, to the pH-induced conformational change of the poly(L-aspartic acid) domain.

Thus, the membrane from butyl methacrylate–L-aspartic acid graft copolymer may be regarded as a good model of biomembrane; the transmembrane polypeptide domain undergoes the pH-dependent reversible conformational change, with impermeable poly(butyl methacrylate) domain as the stable matrix.

Registry No. Poly(β-benzyl L-aspartate) (homopolymer), 25248-99-1; poly(β-benzyl L-aspartate) (SRU), 25736-41-8.

(11) Kondo, Y.; Iizuka, E.; Oka, A.; Hayakawa, T. *Polymer* **1977**, *18*, 111.
(12) Hydrolysis was carried out by immersing the polymer-coated quartz plate into a water–methanol–2-propanol (1:2:2, by volume) mixed solvent containing 5 wt. % NaOH for 16 h.
(13) Brahms, J.; Spach, G. *Nature (London)* **1963**, *200*, 72.
(14) The graft copolymer used in the transport experiment was that having the degree of branching of 16 mol % and $\overline{DP}_n$ of the branch of 10, 0.12 g of which was blended with 0.15 g of homopolymer of butyl methacrylate (M_r 40 000) in order to reinforce the membrane. The mixture (0.27 g) was dissolved in chloroform (10 mL) and was cast on a Teflon-brand plate (diameter, 5 cm). The membrane (ca. 0.2 mm thick) obtained by solvent evaporation was fixed vertically in the center of a diaphragm cell. Hydrolysis of the membrane was performed by filling the both sides of the cell with a NaOH solution[12] for 16 h.

Direct Evidence for Bridge–Terminal Carbonyl Exchange in Solid Dicobalt Octacarbonyl by Variable-Temperature Magic Angle Spinning ^{13}C NMR Spectroscopy

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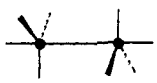
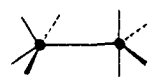
Structural problems in metal carbonyl chemistry have historically been difficult to solve. Perhaps the best documented example is the solid-state structure of $Fe_3(CO)_{12}$.[2-4] High-resolution solid-state ^{13}C NMR spectroscopy has contributed further to the understanding of this molecule by elucidating a fluxional process in the solid state.[5] In general, high-resolution NMR spectroscopy of solids has proven to be an important tool for characterizing solid-state dynamics.[6,7]

For dicobalt octacarbonyl, previous structural investigations have dealt mainly with the solution structure. In a series of elegant papers, several research groups demonstrated, by infrared[8-10] and Raman[11] spectroscopy, the presence of three isomers for $Co_2(CO)_8$ in solution and in frozen matrices, Ia–c. One of these, Ia, represents the molecular structure as determined by X-ray crystallography.[12] In solution, these isomers interconvert rapidly, resulting in the exchange of bridging and terminal carbonyl ligands.

Ia 

Ib 

Ic 

Previously, one of us had reported the observation of a single resonance in the high-resolution ^{13}C NMR spectrum of solid $Co_2(CO)_8$ at room temperature.[13] The implied dynamic behavior,

(1) (a) Virginia Polytechnic Institute and State University. (b) JEOL, USA, Inc.
(2) Wei, C. H.; Dahl, L. H. *J. Am. Chem. Soc.* **1966**, *88*, 1821.
(3) Cotton, F. A.; Troup, J. M. *J. Am. Chem. Soc.* **1974**, *96*, 3070.
(4) Desiderato, R.; Dobson, G. R. *J. Chem. Educ.* **1982**, *59*, 752.
(5) Dorn, H. C.; Hanson, B. E.; Motell, E. *Inorg. Chim. Acta* **1981**, *54*, L71.
(6) Lyerla, J. R.; Yannoni, C. S.; Fyfe, A. A. *Acc. Chem. Res.* **1982**, *15*, 208.
(7) Szeverenyi, N. M.; Bax, A.; Maciel, G. E. *J. Am. Chem. Soc.* **1983**, *105*, 2579.
(8) Bor, G.; Noack, K. *J. Organomet. Chem.* **1974**, *64*, 367.
(9) Bor, G.; Dietter, U. K.; Noack, K. *J. Chem. Soc., Chem. Commun.*, **1976**, 914.
(10) Sweany, R. L.; Brown, T. L. *Inorg. Chem.* **1977**, *16*, 415.
(11) Onaka, S.; Shriver, D. F. *Inorg. Chem.* **1976**, *15*, 915.
(12) Sumner, G. F.; Klug, H. P.; Alexander, L. E. *Acta Crystallogr.* **1964**, *17*, 732.
(13) Dorn, H. C.; Hanson, B. E.; Motell, E. *J. Organomet. Chem.* **1982**, *224*, 181.

0002-7863/84/1506-0251$01.50/0