The N-terminal sequence of the 5K peptide is Pro-Glu-Leu-Leu-Tyr-Ala-, which is uniquely located at residues 151–156 of BSA.[4] The C-terminus of the 5K peptide is -Lys-Val-Leu-Thr-Ser, uniquely located at positions 186–190.[4] Thus the 5K peptide was produced by two peptide bond cleavages: one between Ala-150 and Pro-151, and the other after Ser-190, giving a 40 amino acid peptide having M_r 4535.

The N-terminal sequence of the 45K fragment is Ser-Ala-Arg-Gln-Arg-Leu-, which is uniquely located at residues 191–196.[4] The C-terminal sequence of the 45K fragment is -Gln-Thr-Ala-Leu-Ala, identical with the *C*-terminal sequence of native BSA.[4] Thus, cleavage occurred between residue Ser-190 and Ser-191, giving a 392 amino acid fragment having M_r 43821 and containing the C-terminus of BSA.

Remarkably, the amino acids at the sites of cleavage are detected unaltered; the sequences of the fragments exactly match that of the parent protein, with no gaps. For standard sequencing procedures, the cleavage has the same result as cleavage by a proteolytic enzyme. However, the *yield* of the N-terminal amino acid in the first cycle of Edman degradation is roughly half that of the following residue obtained in the second cycle, implying that other reactions besides hydrolysis accompany the production of the new N-terminus. The extent of degradation of amino-acid side chains was explored by comparing the experimentally determined amino acid composition of one cleaved peptide (17K) with the expected composition.[11] Loss of natural amino acids was slight; in the worst case, the histidine content was 83% of theoretical.

The observed cleavage at both Ala-Pro and Ser-Ser shows that this process does not depend strongly on the chemical reactivity of the amino acid residue which is to be cleaved. Since the peptide bond between the other Ser-Ser sequence in the protein (Ser-270 and Ser-271) was not affected, cleavage appears to depend on proximity of the reagent to the polypeptide chain (no crystal structure is available for comparison).

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(11) The 17K peptide was hydrolyzed in 6 N HCl for 24 h at 110 °C. Trp, Cys, and Met were not determined.

Electrochemical Synthesis of Ultrathin-Film Composite Membranes

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Membrane-based separations are often less energy intensive and more resource conservative than alternative separation methods.[1] Synthetic membranes have, therefore, been the focus of considerable recent research effort.[1] The development of ultrathin-film composite membranes was one of the most important breakthroughs in the synthetic-membrane area;[1b,2] these membranes consist of a porous support layer and a dense, ultrathin[2] active layer. The porous support layer provides mechanical strength yet is highly permeable. The separation process occurs primarily in the ultrathin active layer; because this layer is thin, the overall flux of permeate through the membrane is high. Thus, composite membranes can provide good mechanical strength, high selectivity, and high permeability. This combination of attributes usually cannot be obtained with homogeneous membranes.[1b,3]

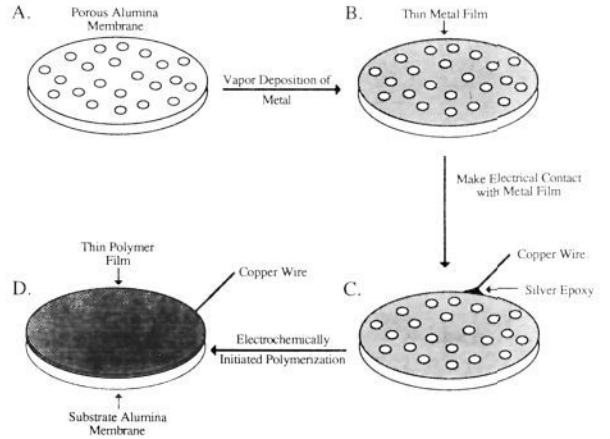

Figure 1. Schematic of ultrathin-film composite membrane fabrication procedure.

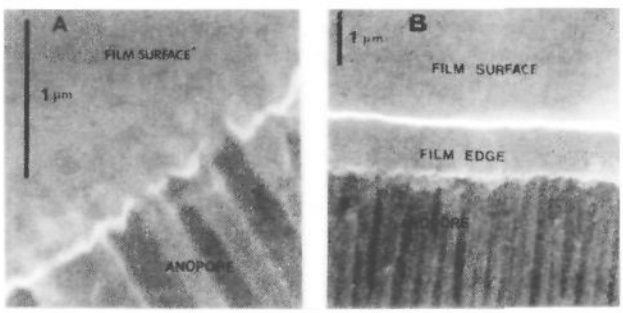

Figure 2. Electron micrographs of cross sections of two ultrathin-film composite membranes.

We have developed a new method for preparing ultrathin-film composite membranes. This method involves electrochemically initiated polymerization at a microporous support-membrane surface and yields an ultrathin[2] polymer film on one face of the support membrane. We describe the synthesis, and preliminary results of electrochemical characterization, of such membranes in this communication.

Figure 1 shows a schematic of the procedure used to prepare the ultrathin-film composites. Anopore (Alltech) Al_2O_3 filters were used as the support membranes;[4] Anopore is 65% porous and contains linear, cylindrical, 200-nm-diameter pores. The Anopore surface is first coated with a ca. 50-m layer of gold;[5] this layer is too thin to block the pores (Figure 1B). A copper wire is attached, and the resulting electrode (Figure 1C) is immersed into a solution containing the electropolymerizable monomers. Electropolymerization causes a thin polymer skin to "grow" over the Anopore surface (Figure 1D).

Our work to date has focused on copolymers of divinylbenzene (DVB) and ethylvinylbenzene (EVB). These monomers can be reduced electrochemically to anions which polymerize via a conventional anionic mechanism.[6] Technical grade DVB (55% DVB and 45% EVB, Polysciences) was extracted with 10% NaOH to remove polymerization inhibitors. The extract was washed three times with purified water. The DVB/EVB was then passed through a column of activated alumina; the effluent was stored (in the dark) over calcium hydride at −5 °C. Polymerization solutions were prepared by mixing measured volumes of the purified DVB/EVB with *N*,*N*-dimethylformamide (DMF);[7] solutions

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(4) Furneaux, F. C.; Rigby, W. R.; Davidson, A. P. *Science* **1989**, *337*, 147.

(5) Au layers were deposited by using a commercial Ar plasma coater.

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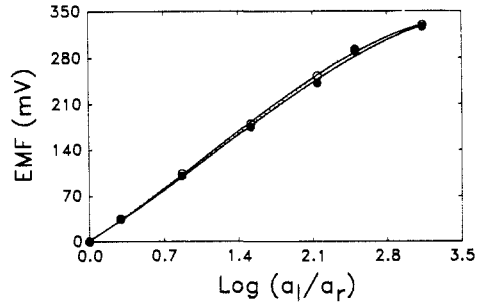

Figure 3. Plot of cell potential (see eq 1) vs log of ratio of the activities of Na^+ on either side of the membrane. Concentration of NaCl on the left side was 2 mM; concentration on the right side was varied between 2 mM and 5 M. ●: Nafion membrane. ○: ultrathin-film composite membrane; film thickness was ca. 1.5 μm.

were also 0.2 M in Bu_4NClO_4, which served as the supporting electrolyte.[7]

The electropolymerization cell contained the Au/Anopore working electrode, an Ag wire quasi-reference, and a Pt foil counter electrode. The polymerization solution was vigorously degassed with purified Ar. Polymerization was initiated by scanning the working electrode potential (200 mV s^{-1}) once from 0 to −2.75 V and back.[8] The voltammetric wave consisted of a single cathodic peak, with no anodic return wave. The poly(DVB/EVB) film which had formed across the membrane surface was rinsed with copious quantities of acetone and air-dried.

Figure 2 shows electron micrographs of cross sections of typical ultrathin-film composite membranes. The poly(DVB/EVB) films are uniformly coated across the Anopore support-membrane surfaces and have uniform film thicknesses.[9] Film thickness was approximated from such micrographs. Films with thicknesses ranging from 3.0 μm to 100 nm have been prepared via this method.[10] The chemical identity of the poly(DVB/EVB) films was established by using Fourier transform infrared spectroscopy.[11]

We have used gas-transport,[1b,c] voltammetric, and potentiometric[12] experiments to prove that the composite membranes are defect-free. Only the potentiometric measurements will be discussed here. Potentiometric data were obtained from sulfonated[13] versions of the poly(DVB/EVB)-based composites. The following cell was employed:

Ag|AgCl|(NaCl, 2 mM)|composite|(NaCl, 2 mM–5 M)|Ag|AgCl

The potential of this cell is given by[12]

$$E_{cell} = \left(2t + \frac{RT}{F}\right) \ln (a_r/a_l) \qquad (1)$$

where t_+ is the transference number for Na^+ in the membrane, and the a terms are the Na^+ activities. If the poly(DVB/EVB-SO_3^-) films are defect-free (and cation permselective), the composite membranes will show cation transference numbers of unity.

Figure 3 shows potentiometric data, plotted according to eq 1; the dotted curve was calculated with the assumption that $t_+ = 1.0$. The experimental data are for an 1100 equiv wt Nafion[14] and an ultrathin-film composite membrane. Nafion is one of the most cation permselective materials known.[14] This is reflected in the enormous concentration range over which the Nafion data fall on the $t_+ = 1.0$ line. Remarkably, the ultrathin-film composite membrane data are essentially identical with the Nafion data (Figure 3). These data indicate that the composite membranes are permselective and defect-free.

In closing, it is worth noting that the method described here should be applicable to any of the vast number of materials that can be synthesized electrochemically.[15] Other microporous support membranes could also be employed.

Acknowledgment. We acknowledge invaluable discussions with Drs. David Moll and Richard Fibiger of the Dow Chemical Company. This work was supported by the Air Force Office of Scientific Research, The Office of Naval Research, and the Dow Chemical Company.

(7) DMF was dried over CaH_2 prior to use. Bu_4NClO_4 was recrystallized from ethyl acetate/pentane and dried in vacuo.

(8) Potentials are reported vs Ag quasi-reference. Positive feedback was employed to correct for uncompensated solution resistance. Martin, C. R.; Rubinstein, I.; Bard, A. J. *J. Electroanal. Chem.* **1983**, *151*, 267.

(9) Low-quality films showed "rainbow-like" optical diffraction patterns. High-quality films, which were hundreds of nanometers thick, showed only one color.

(10) Film thickness was varied by varying the monomer concentration. The thinnest films were prepared on a version of Anopore which contains 200-nm-diameter pores which branch at one surface into 20-nm-diameter pores. A complete report is in progress.

(11) FTIR spectra showed peaks at 795 and 833 (meta-disubstituted phenyl ring) and 1512 and 1481 (para-disubstituted phenyl rings) cm^{-1}.

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Mechlorethamine Cross-Links Deoxyguanosine Residues at 5′-GNC Sequences in Duplex DNA Fragments

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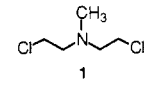
Mechlorethamine (**1**, *N*-methylbis(2-chloroethyl)amine) is the simplest of the nitrogen mustards. The ability of nitrogen mustards to cross-link the two strands of duplex DNA[1] is widely believed to account for their antitumor activity.[2] Isolation from hydrolysates of mechlorethamine-treated DNA of the conjugate[3] **2** first led to the reasonable suggestion that deoxyguanosine residues of adjacent base pairs are preferentially cross-linked,[3,4] a notion which persists.[5] We report here that mechlorethamine cross-links duplex DNA fragments through the distal deoxyguanosine residues at the sequence 5′-GNC (N=G or C) in preference to 5′-GC or 5′-CG sequences.



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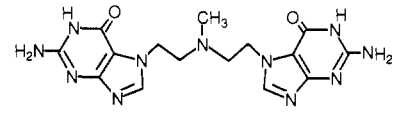

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Self-complementary DNAs I and II (Figure 1) were treated with **1**.[6] Analysis of the product mixtures by denaturing poly-

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(6) DNA was synthesized (Applied Biosystems Model 380A) and was purified by denaturing PAGE (20%, 25:1 acrylamide/bisacrylamide, 40% urea) and radiolabeled.[7,8] DNAs (0.75 OD/100 μL 250 mM aqueous sodium cacodylate (pH 8)) were incubated 1 h at 37 °C with **1** (2.5 mM for I, II; 0.25 mM for III). Cross-linked DNA was isolated by ethanol precipitation, resuspension in 5 M urea, and 20% denaturing PAGE (19:1 acrylamide/bisacrylamide, 50% urea, 0.35 mm thick, 41 × 37 cm). Piperidine cleavage products were evaluated by PAGE, gel drying, and densitometry;[8] bands were assigned by reference to a Maxam–Gilbert G-lane.[10]

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