and we are presently conducting such studies. With this information, we expect that the present strategy using Au/Ag(upd) electrodes for halide detection will provide the notable abilities to measure the concentrations of multiple halides simultaneously and to provide specific signals for confirming their identification.

Experimental Section

Materials: Au (99.99%) shot and Cr-coated tungsten filaments were obtained from Americana Precious Metals (East Rutherford, NJ) and R. D. Mathis (Long, Beach, CA), respectively. Sulfuric acid (double distilled, 98%) and silver sulfate ($c(Cl^-) \leq 0.02\%$) were obtained from Aldrich and used as received. KCl, KBr, and KI were obtained from Mallinckrodt and used as received. Au(111) samples were prepared by sequential evaporation of Cr (2–3 nm) and Au (150 nm) onto glass slides and a post-evaporation annealing in a hydrogen flame.[12]

Electrochemical measurements: Cyclic voltammetry was conducted with a computer-controlled PAR Model 263A potentiostat. A solution of 0.6 mM Ag_2SO_4 and 0.1M H_2SO_4 in deionized water (Millipore, 18.2 MΩ) was used to deposit the Ag upd adlayer on gold. Modified electrodes were prepared by cycling once in this solution and being removed at a 300 mV versus Ag^+/Ag^0 under potentiostatic control.[13] The Au(111)/Ag(upd) electrodes were rinsed with deionized water and immersed into a halide test solution for varying amounts of time. The samples were rinsed with water and characterized electrochemically in a 0.6 mM Ag_2SO_4/0.1M H_2SO_4(aq) solution. A standard three-electrode configuration was used to obtain all CVs, and all potentials are quoted relative to silver wire (Ag^+/Ag^0).

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[9] The detection limit of a chloride-ISE is 50 μM.[3]
[10] This ability is a notable advantage over other methods (such as ISEs) that provide a weighted aggregate view of ion concentration.
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Mid-Membrane Photolabeling of the Transmembrane Domain of Glycophorin A in Phospholipid Vesicles**

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The topography of membrane-bound proteins at atomic resolution is known only in rare cases.[1] Although the primary amino-acid sequence of glycophorin A (GPA), the major sialoglycoprotein of the human erythrocytes, has been known for more than twenty years and was the first membrane protein sequence elucidated,[2] a three-dimensional picture of the protein is still missing. We have previously developed the photoactivable membrane probe **1**. This is a phospholipid with two distal, polar heads (a bola-amphiphile) and carries a photosensitive group (benzophenone) in the middle of a transmembrane chain. It is easily incorporated into DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocoline) vesicles, where it spans the bilayer at least in the presence of physiological concentrations of cholesterol. We demonstrated recently that the tandem use of the probe **1a** and cholesterol (for its ordering effect) in photolabeling experiments on DMPC vesicles led to a remarkable regioselective functionalization

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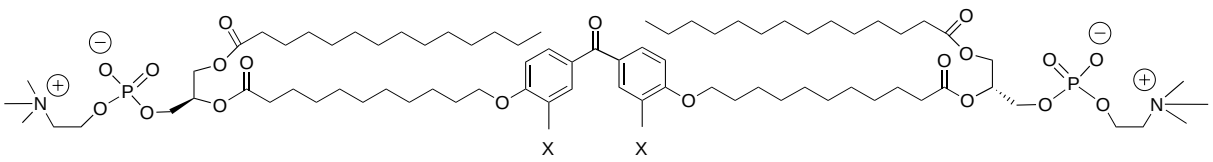

1a (X=H), **1b** (X=^{2}H), **1c** (X=^{3}H), **1d** (X=I)

of the ω-1 and ω-2 positions of the surrounding phospholipidic chains and the C-25 position on the side chain of cholesterol.[3]

Probe **1** has now been used to explore the in situ topography of the transmembrane domain of GPA. GPA consists of a single polypeptide chain of 131 amino acid residues ($M_w \approx 31$ kDa) and has been shown to have a tripartite structure: The extra-cellular N-terminal domain in which 16 amino acids are glycosylated, the hydrophobic transmembrane domain of about 20 amino acids, and the hydrophilic C-terminal domain exposed to the cytosol. Engelman's group showed, by a fluorescence energy-transfer method, that the transmembrane domain of GPA containing residues 62–100/101 (Figure 1), forms a noncovalent dimer in phospholipid

Figure 1. Sequence of the glycophorin A transmembrane domain and its vicinity: The hydrophobic region[4, 6] is underlined; the residues at the dimer interface are in bold. Stars indicate amino acids photolabeled with probe **1c**.

bilayers.[4] They also proposed a model for the three-dimensional structure of the dimeric, transmembrane domain of GPA by NMR studies of residues 73–96 of GPA in micelles: a right-handed supercoil of α-helices with a -40° crossing angle.[5] A dimerization motif, composed of Leu75, Ile76, Gly79, Val80, Gly83, Val84, and Thr87, was identified by mutagenesis studies[4] and by rotational-resonance NMR studies (Figure 1).[6]

In this Communication, we describe the analytical process that led us to the identification of the photolabeled amino acids of GPA. The different steps are a) proteoliposome reconstitution, b) photoirradiation, c) trypsin digestion, d) purification of the peptide fraction, and e) Edman degradation. Firstly, proteoliposomes were prepared by a detergent-mediated method[7] (with *n*-octylglucoside (0.1M) as detergent), from DMPC (61 mol%), DMPS (1, 2-dimyristoyl-*sn*-glycero-3-phosphoserine, 5.0 mol%), cholesterol (33 mol%), radioactive probe **1c** (70 mCi, 0.01 mol%), and GPA (0.2 mol%).[8] Sucrose density-gradient centrifugation revealed that the proteoliposomes thus obtained comprise only one fraction containing phospholipids (65 mol%), cholesterol (34 mol%), GPA (0.16 mol%), and probe (0.01 mol%). These proteoliposomes were irradiated at 365 nm for 10 min, then treated with trypsin (37 °C, 18 h) to cleave the peptide chain exposed to the exterior of vesicles. Ultracentrifugation of the suspension obtained could separate the precipitate containing the proteoliposome fraction from the aqueous supernatant containing the cleaved small peptide fragments. The precipitate was then extracted twice with cold ethanol and centrifuged to remove the lipidic components. The remaining peptidic fraction was analyzed by SDS-PAGE (15% acrylamide). Peptide fragment TC (transmembrane fragment plus C-terminal fragment, residues 62–131)[9] was extracted from the gel and subjected to Edman degradation. The radioactivity associated with the peptide fragment TC corresponds to 5% of the total radioactivity of the starting proteoliposomes. Analysis of the distribution of the radioactivity along the sequence showed that it is restricted to Val80 and Met81 residues (Figure 2). On the other hand, the

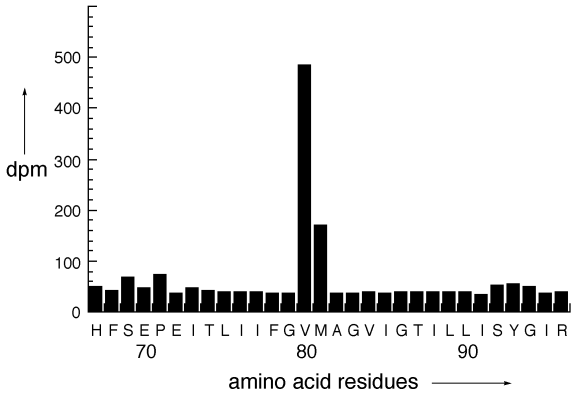

Figure 2. Edman degradation of peptide fragment TC (residues 62–131), and the distribution of radioactivity. The *y* axis corresponds to the radioactivity (dpm) along the sequence H67 to R96.

orientation of GPA in reconstituted proteoliposomes is very likely to be the same as that in natural erythrocyte membranes, that is, the N-terminal is exposed to the outside of vesicles, since only one dimer of about 18 kDa ($2 \times$ residues 62–131) was detected by Coomassie-blue staining on a SDS-PAGE gel after digestion of the proteoliposomes with proteinase K.[9]

These results (Figure 2) show that Val80 has been highly regioselectively photolabeled in addition to Met81. Since probe **1b** has been confirmed by a solid-state ^{2}H NMR to be incorporated into DMPC vesicles, in a transmembrane manner, in the presence of 33 mol% of cholesterol[3] and since the transmembrane domain of GPA (residues 73–95 or 71–99) has been shown by ATR-FTIR measurements to be perpendicular to the bilayer surface in DMPC vesicles by Ruysschaert's[9] and Smith's[10] groups, the results show that Val80 and Met81 are localized in the center of the bilayer. Therefore, our results are in a good agreement with the structural features of the transmembrane domain of GPA in membranes, which have been proposed earlier.[4–6, 9, 10] This represents a marked improvement over the results described earlier. For example, Khorana's group[11] had tried to use, for

the same goal, phospholipidic probes carrying a photoactivable carbene precursor on the ω-position of the fatty-acid chain. However, most of the crosslinking involved Glu 70, which should be situated at the lipid–water interface in vesicles.[9] Their results might reflect the extensive disorder of the phospholipidic matrix and of the probe itself above the phase-transition temperature. In our case, the formation of an ordered bilayer structure due to the transmembrane "immobilization"[3, 12, 13] of the probe and to the concomitant use of cholesterol has led to an excellent selectivity for the photolabeling on the transmembrane domain of GPA in vesicles.

As judged by Engelman's three-dimensional model for the dimeric transmembrane domain of GPA, Ala 82 could also be potentially crosslinked by the probe. There could be two possible explanations for the lack of radioactivity associated with Ala 82. Either Phe 78 could generate a steric hindrance as neighbor residue on the preceding α-helical turn or, alternatively, a photochemical reaction had occurred on Ala 82 but a retro-aldol type degradation of the α-coupling product formed might have followed. Such a degradation of α-coupling products of amino acid derivatives, especially in the case of alanine, has been observed by us[14] and had already been noted by Schöllkoff et al.[15] In contrast, methionine is functionalized at positions (CH_3 and CH_2) located α to the sulfur atom.[14, 16] Valine should be attacked at the β-position ($(CH_3)_2CH$) in lipid bilayers. Since the radius of the reactive sphere for a benzophenone is estimated to be 3 Å around the carbonyl oxygen atom,[17] the α-position of valine could not be attacked. Therefore, no such degradation was observed in the case of the valine or methionine adducts.

For the first time, to our knowledge, the center of the transmembrane domain of a protein has been selectively functionalized. Although *photoaffinity-labeling* methods have been successfully employed to determine receptor–ligand binding sites,[18] there has been no success so far in the site-selective photolabeling of proteins *within* phospholipid bilayers. Despite the limitation cited above for the use of our probe, this method could be useful for the study of transmembrane domains of other proteins.

Experimental Section

Probe **1c** was synthesized from the diiodo precursor **1d**, according to the procedure employed for the synthesis of the dideuterated analogue **1b**[19] except for the use of $^3H_2$ in place of $^2H_2$. For the tritium-labeled compound **1c**, the radiochemical purity was checked by thin-layer chromatography on silica gel and $CHCl_3$/MeOH/H_2O (65/25/4) eluent and by HPLC using a Zorbax NH_2 column (eluent MeCN/MeOH/phosphate buffer pH 4.8 (50/40/10)). The former method showed the purity to be 100%; the latter 98.6%. **1c**: Specific radioactivity 40 Ci mmol^{-1}; ^{3}H NMR (320 MHz, $CDCl_3$/CD_3OD (9/1)): $\delta = 6.90$ (single sharp peak); UV/Vis (MeOH): λ_{max} (ε) = 200 (30000), 223 (14500), 295.5 nm (22500 M cm^{-1}). **1c** was stored as a stock methanolic solution at $-20\,^{\circ}C$.

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A Direct and Efficient α-Selective Glycosylation Protocol for the Kedarcidin Sugar, L-Mycarose: $AgPF_6$ as a Remarkable Activator of 2-Deoxythioglycosides**

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Without the stereodirecting ability of 2-substituents, strategies to prepare 2-deoxyglycosides selectively in high α- or β-anomeric forms rely heavily on indirect sequences from glycals or latent 2-deoxysugars.[1–3] As such, these require a subsequent reductive step that would be unsuitable for many

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