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CHARACTERIZATION OF THE AMPHIPATHIC STRUCTURE OF γ-GLUTAMYLTRANSPEPTIDASE F13

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Rat renal γ-glutamyltranspeptidase (γGT) is an intrinsic membrane glycoprotein which is composed of two nonidentical subunits. The hydrophobic domain responsible for its membrane interaction is associated with the large subunit. Amphipathic (TγGT) and hydrophilic (PγGT) forms of γGT were purified from brush border membranes following Triton X-100 solubilization and papain proteolysis, respectively (1). Amino acid analysis of the separated TγGT and PγGT subunits revealed no significant differences in the composition of the small subunits. However, the large subunit of the TγGT contained an additional 150 residues, 56% of which were hydrophobic. Only the TγGT form can be incorporated into unilamellar phosphatidylcholine (PC) vesicles (2). Reconstitution provided a convenient means to protect the hydrophobic domain from proteolytic degradation and to facilitate its isolation.

RESULTS AND DISCUSSION

Fully active, ^{3}H-labeled TγGT was prepared by reductive methylation (3) and incorporated into [^{14}C]PC vesicles. Reconstitution was evident from the coelution of [^{3}H]TγGT and the [^{14}C]PC vesicles observed during chromatography on Sepharose 4B (Fig. 1). The TγGT-vesicle association is unaffected by treatment with TPCK-trypsin, *Staphylococcus aureus* V8 protease, or thermolysin. In contrast, incubation with papain causes the release of 85–95% of the γGT activity. During Sepharose 4B chromatography, the solubilized activity elutes with an elution volume characteristic of PγGT (Fig. 1). The fractions containing the [^{14}C]PC vesicles retain 30–40% of the ^{3}H-labeled protein but only 5–15% of the γGT activity. ^{3}H-labeled material also elutes in the retention volume (V_R) of the column. When [^{3}H]PγGT and [^{14}C]PC vesicles are preincubated with papain and then chromatographed, no ^{3}H label elutes with the vesicles or in the V_R.

The subsequent fractionation of the vesicle-associated material was monitored by sodium salicylate fluorography of samples subjected to electrophoresis on a 20% polyacrylamide slab gel. As shown in Fig. 2, reductive methylation incorporates ^{3}H label into both subunits of TγGT (lane 1). The vesicle-associated protein obtained following papain treatment of reconstituted [^{3}H]TγGT is a mixture of residual [^{3}H]TγGT and a newly generated ^{3}H-labeled peptide which migrates slightly slower than the dye front (lane 2). The residual [^{3}H]TγGT and the ^{3}H-labeled peptide were resolved by chromatography on Sephadex LH-60 in organic solvents. The residual amphipathic [^{3}H]TγGT is not retained by the resin (lane 3), whereas the hydrophobic ^{3}H-labeled peptide is partially adsorbed and is recovered with a greater elution volume (lane 4). Fluorescamine and ^{3}H radioactivity profiles of the LH-60 column fractions were coincident, indicating the absence of any unlabeled peptides.

The ^{3}H-labeled material isolated from the V_R fractions of the Sepharose 4B column was further resolved by Sephadex G-25 chromatography in 8 M acetic acid. This fraction contains at least three fluorescamine positive peptides, two of which were ^{3}H-labeled. The fractionated peptides ranged between 400 and 1,200 in mol wt. Dansyl-Cl analysis failed to detect the presence of free amino acids. Each of the peptides is apparently a unique

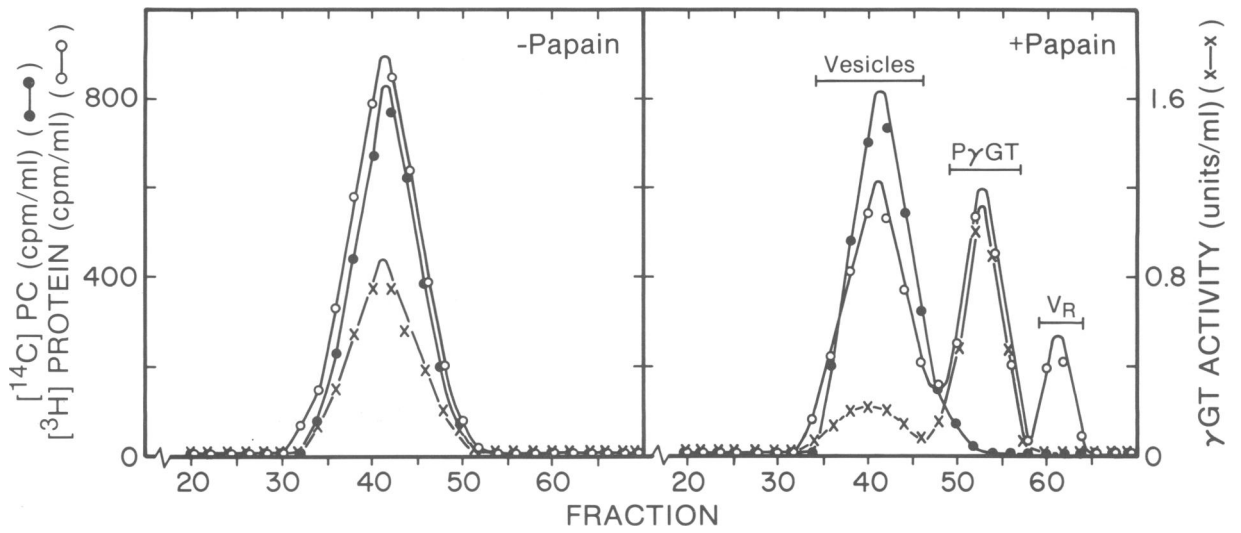

FIGURE 1 Sepharose 4B chromatography of ^{3}H-labeled TγGT reconstituted into [^{14}C]PC vesicles and preincubated either in the absence or presence of a 40:1 molar ratio of TγGT:papain. For preparative purposes unlabeled PC was used.

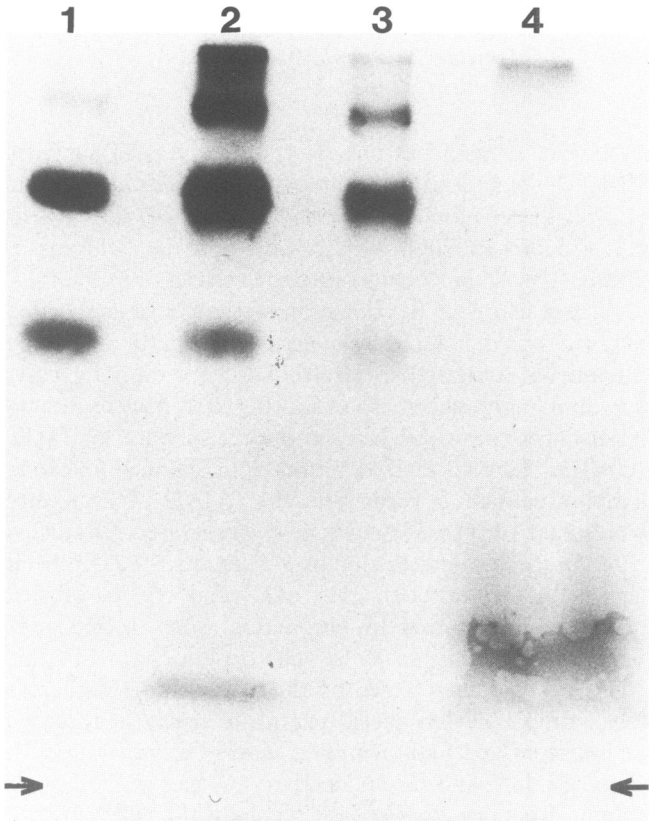

FIGURE 2 Analysis of the fractions obtained during the isolation of the membrane binding domain of TγGT. The ^{3}H-labeled fractions were subjected to electrophoresis on a 20% polyacrylamide slab gel containing 6 M urea and 0.1% sodium dodecyl sulfate. The fractions were visualized by sodium salicylate fluorography. The various lanes contain: 1, ^{3}H-labeled TγGT; 2, the vesicle-associated material pooled from the Sepharose 4B column; 3, residual, vesicle-associated [^{3}H]TγGT not retained by Sephadex LH-60; lane 4, vesicle-associated ^{3}H-labeled peptide retained by Sephadex LH-60. The arrows denote the bromophenol blue dye front.

fragment since reincubation of the larger peptides with papain did not increase their elution volume. Due to their small size, the Sepharose 4B V_R peptides were not detectable by fluorography following polyacrylamide gel electrophoresis.

The vesicle-associated ^{3}H-labeled peptide purified by Sephadex LH-60 chromatography and the unfractionated peptides which elute in the V_R of the Sepharose 4B column were used for the following studies. The relative hydrophobic/hydrophilic character of the two fractions was analyzed by determining their ability to partition between an aqueous phase and a series of primary aliphatic alcohols ranging from 1-butanol to 1-decanol. In each case the peptide purified by LH-60 chromatography quantitatively partitioned into the organic phase, whereas the V_R peptides quantitatively partitioned into the aqueous phase. In addition, only the LH-60 peptide could be reconstituted into PC vesicles. The association of the reconstituted ^{3}H-labeled peptide with the vesicles was unaffected by treatment with 1 M NaCl or by reincubation with papain. Therefore this association is nonionic and may closely approximate the orientation of [^{3}H]TγGT within the lipid bilayer. It is evident from these experiments that the V_R peptides are hydrophilic in character, whereas the LH-60 peptide is hydrophobic and retains the ability to interact with a lipid bilayer. However, amino acid analysis indicated that the content of hydrophobic residues of the V_R peptides is slightly greater than that determined for the LH-60 peptide (53% vs. 46%). Therefore, the relative hydrophobic/hydrophilic character of the two fractions must be due to the combined contributions of primary and secondary structure and not simply to their relative content of hydrophobic residues.

Papain proteolysis of TγGT reconstituted into a lipid

bilayer results in the generation of a vesicle-associated hydrophobic peptide and the release of a mixture of hydrophilic peptides. The former peptide probably constitutes the hydrophobic domain which serves to anchor the native γGT to the renal brush border membrane. The hydrophilic peptides may be derived from the sequence of amino acids which connects the separate domains of this amphipathic protein.

Received for publication 4 May 1981.

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STRUCTURE OF THE H^+-ATPASE ($F_1 \cdot F_0$) FROM *ESCHERICHIA COLI* AND ITS DICYCLOHEXYLCARBODIIMIDE-PROTEIN IN SOLUTION BY X-RAY SCATTERING METHODS

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The H^+-ATPase ($F_1 \cdot F_0$) synthetase of energy-transducing membranes couples the vertical movement of H^+ through the membrane to synthesize ATP (1). The F_1 portion, having a molecular weight of 365,000 (2) can be released from membranes at low ionic strength. The F_0 part, which is the integral membrane portion, renders dicyclohexylcarbodiimide (DCCD) sensitivity (3). Heavy atom labeling of the F_1-portion with tetrakis (acetoxymercuri) methane (4) (TAMM) or mercuriated *N*-pyrrolo-isomaleinimide (5) (NEM-Hg), which carries four Hg^{2+}, was applied on F_1 and $F_1 \cdot F_0$. The localization of the Hg label was determined from the distance distribution function, D_{PL}, by measuring the scattering curves of $F_1 \cdot F_0$ and F_1 with and without the label, and measuring the scattering intensities of the unbound Hg^{2+} label ($I_L[h]$). $I_L(h)$ was automatically subtracted from the difference of the scattering curves of labeled and unlabeled $F_1 \cdot F_0$. The distribution of distances between the label and any position in the $F_1 \cdot F_0$ structure is given through D_{PL} and enables us to locate the heavy atom level.

RESULTS AND DISCUSSION

The Hg_4^{2+}-label peaks at R=18 Å only, whereas the maximum chord length was determined to be 174 ± 10 Å for $F_1 \cdot F_0$; for $F_1 \cdot F_0$-Hg_4^{2+} it was 180 ± 10 Å; and for F_1 it was 125 ± 10 Å. The morphological parameters of the labeled and unlabeled complexes are listed in Table I. No severe changes in volume or radii of gyration are detected, indicating that size and shape heterogeneities are absent. Furthermore, the biological activity of $F_1 \cdot F_0$ as well as of F_1 is not affected by the heavy atom label, indicating that the SH group, which is attainable from the outside of $F_1 \cdot F_0$ is involved neither in the ATP hydrolysis, nor in the catalysis of the $^{32}P_i$-ATP exchange rate.

When the F_1 portion was labeled with TAMM or NEM-Hg, the distance distribution function peaked at 31 ± 5 Å, without any change in D_{max}=125 ± 10 Å. Chemical analysis of the location of the Hg_4^{2+} cluster revealed that Hg_4^{2+} is located on the β-subunit of F_1; however, for the $F_1 \cdot F_0$ complex the label is situated on the α-subunit. Possibly, a molecular rearrangement of the five subunits of F_1 occurred when F_0 assembled to the DCCD-sensitive H^+-ATPase. A second Hg_4^{2+} cluster can be inserted into the F_1 complex, located at D_{PL} = 50 Å which is not possible with the $F_1 \cdot F_0$ complex. Methyl-mercury nitrate (CH_3HgNO_3) can be bound to the tail end of the distribution function of $F_1 \cdot F_0$, revealing a profile of $D_{PL}(R)$ of $F_1 \cdot F_0$ along its major axis of a = 85 Å. The distance between the CH_3-$HgNO_3$ label, situated in the F_0 portion, and the Hg_4^{2+} cluster was evaluated at 140 ± 10 Å.

The DCCD-protein involved in the H^+-transmembrane channeling process undergoes a reversible water-induced transformation from α-helical to β-structure. The funda-