Warfarin Resistance Is Associated with a Protein Component of the Vitamin K 2,3-Epoxide Reductase Enzyme Complex in Rat Liver

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Summary

Warfarin, the most used drug in the world in long-term anticoagulation prophylaxis, targets the vitamin K 2,3-epoxide reductase (VKOR) of the vitamin K cycle in liver. Recently, the enzyme has been identified as a multicomponent lipid-protein enzyme system in the endoplasmic reticulum (ER) membrane (17). As the first step towards understanding genetic resistance to warfarin, we present in this paper data on VKOR from normal and a strain of warfarin resistant laboratory rats maintained in the United States. Metal induced in vitro assembly of the enzyme complex demonstrates that the glutathione-S-transferase (GST) enzyme component of the complex loses its GST activity upon formation of VKOR. Less VKOR activity is measured upon assembly of the complex from warfarin resistant rats. The GST activity measured in warfarin resistant rats, before assembly of the complex, is 10-fold less sensitive to warfarin inhibition than the GST activity measured in normal rats. Microsomal epoxide hydrolase (mEH) is the second component of VKOR. When incubated with the components of VKOR before assembly of the complex, antibodies raised against mEH prevented formation of the enzyme complex. Sequencing of mEH cDNAs from normal and warfarin resistant rats revealed identical sequences. The data suggest that the mutation responsible for genetic warfarin resistance is associated with the GST component of VKOR.

Introduction

Vitamin K1 functions as a cofactor for the vitamin K-dependent carboxylase (γ-carboxylase), an enzyme which resides in the endoplasmic reticulum membrane (ER) and participates in post-translational γ-carboxylation of secretory proteins (1, 2). The carboxylase converts a limited number of glutamic acid residues in the targeted proteins to γ-carboxyglutamic acid (Gla) residues (1, 2). As illustrated in Fig. 1, γ-carboxylation of one Glu residue is coupled stoichiometrically to formation of one molecule of the vitamin K metabolite, vitamin K1 2,3-epoxide. The epoxide is reduced by a liver enzyme(s) to the hydroquinone form of the vitamin which is the active cofactor for the γ-carboxylase. This cyclic conversion establishes a red/ox. cycle for vitamin K in liver, known as the vitamin K cycle (3) (see Fig. 1). Two unrelated vitamin K reducing enzymes have been identified as parts of the cycle (4). The flavoprotein DT-diaphorase (EC. 1.6.99.2) reduces the quinone form of vitamin K1 but can not reduce the epoxide (5) (see Fig. 1). On the other hand, the warfarin sensitive enzyme vitamin K epoxide reductase (VKOR) reduces the epoxide as well as the quinone and thus is essential for operation of the cycle (6).

Because of the immense importance of warfarin as an anticoagulant in prophylactic medicine (7, 8) and the problems with genetic warfarin resistance in humans (9, 10) and rodent pest control (11, 12) numerous attempts have been made to understand the molecular mechanism underlying warfarin sensitive vitamin K epoxide reduction and the genetic basis for resistance (3, 7, 13-15). These studies have been hampered by the difficulties encountered in purification of VKOR.

Recently we have developed a protocol which allows us to purify VKOR 120-fold from the rat liver endoplasmic reticulum (ER) membrane (16). The enzyme has been shown to be a lipid-protein enzyme complex where two unrelated proteins are needed for expression of enzymic activity. Analyses of the protein components have shown that microsomal epoxide hydrolase (mEH) associates with a member of the glutathione-S-transferase (GST) gene family to create the complex in the presence of metal and phospholipid (17). As the first step towards understanding the molecular basis for genetic warfarin resistance we present in this paper data which compare the molecular properties of VKOR in normal rats and a laboratory kept strain of warfarin resistant rats. The data suggest that warfarin resistance is associated with the GST component of the complex.

Materials and Methods

Animals

Normal male Sprague Dawley rats weighing 250-300 grams were purchased from Zivic Miller Laboratories, Inc., Zelienopole, PA. A strain of Rattus norvegicus rats with genetic resistance to warfarin which has been maintained in...
Partial Purification of VKOR

The procedure was carried out as described by Wallin and Guenthner (16) with some modifications. Rat liver microsomes from fasted animals were prepared as described (4) and stored frozen at -85°C. Frozen microsomes derived from 32 g of liver were thawed on ice and suspended in a Dounce homogenizer in 48 ml of 250 mM phosphate, 250 mM KCl, 20% glycerol, 0.75% CHAPS, pH 7.8 (buffer D) containing 5 mM DIFP. The suspension was cleared by centrifugation at 100,000 × g for 30 min and the supernatant made 43% saturated with (NH₄)₂SO₄. After 15 min on ice the precipitate was collected by centrifugation at 10,000 × g for 10 min and dissolved in 16 ml of 0.1 M phosphate, 20% glycerol, 10% (NH₄)₂SO₄, 0.5% CHAPS, pH 7.85 (buffer B) containing 0.4 mg/ml of soy bean lipids which was added to the buffer from a 40 mg/ml stock solution prepared by sonication as described (16). The enzyme solution was cleared by centrifugation at 100,000 × g and loaded onto a 70 × 3.5 cm column of Sepharose 6B equilibrated in buffer B. The void volume fraction, which contains VKOR, was made 0.75% in CHAPS and desalted on a column of Sephadex G-25 in 25 mM phosphate, 25 mM KCl, 20% glycerol, 0.75% CHAPS, pH 7.85 (buffer C) containing 0.2 mg/ml phospholipid. The void volume fraction was sonicated on ice for 5 min using a Branson 250 Ultrasonicator at setting 3. The sonicate was passed through a 0.45 micron filter before it was loaded onto a 6 × 1.5 cm column of hydroxylapatite equilibrated in buffer C. The column was washed extensively with buffer C before the VKOR lipid-protein complex was stepwise eluted from the column with buffer D. The enzyme was stored frozen at -85°C for 3 months with no loss of warfarin sensitive VKOR activity.

Enzyme Assays

Warfarin sensitive VKOR activity was measured as described (6) by estimating % conversion of vitamin K₁, 2,3-epoxide to vitamin K₁. The vitamin and the epoxide were separated by HPLC on a reversed phase C18 column in methanol and quantified against external standards. Vitamin K₁, 2,3-epoxide was the epoxide were separated by HPLC on a reversed phase C18 column in methanol with 0.4 mg/ml of soy bean lipids which was added to the buffer from a 40 mg/ml stock solution prepared by sonication as described (16). The enzyme solution was cleared by centrifugation at 100,000 × g and loaded onto a 70 × 3.5 cm column of Sepharose 6B equilibrated in buffer B. The void volume fraction, which contains VKOR, was made 0.75% in CHAPS and desalted on a column of Sephadex G-25 in 25 mM phosphate, 25 mM KCl, 20% glycerol, 0.75% CHAPS, pH 7.85 (buffer C) containing 0.2 mg/ml phospholipid. The void volume fraction was sonicated on ice for 5 min using a Branson 250 Ultrasonicator at setting 3. The sonicate was passed through a 0.45 micron filter before it was loaded onto a 6 × 1.5 cm column of hydroxylapatite equilibrated in buffer C. The column was washed extensively with buffer C before the VKOR lipid-protein complex was stepwise eluted from the column with buffer D. The enzyme was stored frozen at -85°C for 3 months with no loss of warfarin sensitive VKOR activity.

Extraction of Microsomes with Detergent and Reconstitution of the VKOR Enzyme Complex

Microsomes were resuspended in ice cold 0.025 M imidazole-HCl buffer, pH 7.6 containing 0.5% CHAPS and 5 mM DIFP (buffer A) with 6 strokes in a tight fitting Dounce homogenizer to give a protein concentration of 8 mg/ml. After resuspension, the microsomal homogenate was left on ice for 15 min and then centrifuged at 100,000 × g for 45 min. This extraction procedure was repeated twice to remove contaminating proteins originating from cytosol and the ER lumen. The pellets remaining after extraction were resuspended in buffer A to a protein concentration of 2 mg/ml. Assembly of the components of VKOR in the resuspended extracted microsomes was achieved as described (17) by metal induced lipid aggregation obtained by adding 10 mM MnCl₂ to the test samples. Test samples without MnCl₂ served as controls.

Purification of Membrane Associated GST Enzymes from Detergent Extracted Microsomes

Detergent extracted microsomes were solubilized in 250 mM imidazole, 0.5 M KCl, 0.75% CHAPS, pH 7.8 and diluted 1:1 (v/v) with water prior to chromatography on agarose-GSH equilibrated in buffer A. The column was washed with several bed volumes of buffer A before the retained GST enzymes were eluted with 100 mM GSH in buffer A. Free GSH was removed by dialysis against buffer A.

Immunoadsorption of VKOR from Detergent Extracted Microsomes

Detergent extracted microsomes were prepared as described above and dissolved in 0.1 M HEPES buffer, pH 7.85, containing 0.5 M KCl, 20% glycerol, 0.75% CHAPS and 5 mM DIFP (buffer H). The solution was diluted 1:1 (v/v) with water and then centrifuged for 45 min at 100,000 × g to remove unsolubilized material. To 0.5 ml aliquots of the clear solution in Eppendorf tubes were added 40 µl of mEH antisera, 40 µl of pooled normal control serum and 40 µl of PBS respectively. Prior to these additions 8 µM vitamin K₁, 2,3-epoxide had been added to every sample to protect the epoxide binding site from the mEH antibodies (14). Samples were left on ice over night whereupon 100 µl packed Sepharose-protein A was added to each sample. The Sepharose-protein A particles were allowed to react with the mEH antibodies for 1 h at 4°C by mixing on a tilting platform. The Sepharose particles were washed 4-times by resuspension and centrifugation in buffer H containing 0.2 mg/ml of phospholipids. After the last centrifugation, the particles were resuspended in 100 µl buffer H/phospholipid and each sample tested for VKOR activity. The quantity of protein attached to the Sepharose-protein A beads was determined as the amount of protein released from the particles in 8 M urea. To visualize proteins bound to the Sepharose-Protein A beads, the proteins were released from the beads by treating them with the 8 M urea containing sample solution used for isoelectric focusing in 2-D-SDS-PAGE (20). The proteins were then subjected to 2D-SDS-PAGE and stained with Coomassie blue.

Isolation of RNA and PCR Amplification of cDNA

Poly(A)⁺ RNA was isolated from livers of normal and warfarin resistant rats using the Fast Track mRNA kit from Invitrogen. To obtain a cDNA product for rat mEH the forward (AGGGAACACAGGA) and reverse (GTATCCAGTGTCACTAGG) primers were used to obtain the full length cDNA for mEH from normal and warfarin resistant rats (21). Automatic sequencing was carried out by the DNA Sequencing Core Laboratory at the Bowman Gray School of Medicine.

Protein Sequencing

Protein sequencing of Coomassie blue stained proteins in SDS-PAGE gels was carried out on tryptic peptides by Harvard Microchem, Cambridge, MA. BLAST and FASTA were used to search protein data banks for matching sequences.

Additional Methods

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 10-15% gradient gels was carried out according to Laemmli (22) and stained with Coomassie blue as described (20). Two-dimensional SDS-PAGE (2-D-SDS-PAGE) was carried out as described in (20). For Western blotting, proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA) and immune complexes detected with alkaline phosphatase conjugated secondary antibodies.

Materials

Vitamin K₁, GSH and agaroase-GSH were from Sigma, St. Louis, MO. Asolectin from soybeans was from Fluka, Buchs, Switzerland. A rabbit polyclonal antiserum against rat mEH was a gift from Dr. Thomas Guenthner, Department of Pharmacology, University of Illinois, Chicago.

Results

Partial Purification of VKOR from Normal and Warfarin Resistant Rats

Figure 2 shows 2-D-SDS-PAGE gels of the partially purified VKOR preparations from normal and warfarin resistant rats over a pH range
between 8.0 and 4.5. There were no differences in the protein patterns seen in Fig. 2 whether the VKOR preparation was made from normal rat livers (panel aN) or livers from rats resistant to warfarin (panel bW). Since thiols have been shown to be involved in warfarin binding to VKOR (11) we also identified the proteins in the two preparations which could be labeled with the thiol specific reagent 3H-NEM. Previous experiments have shown that NEM is an inhibitor of VKOR (23). Fluorograms of the 3H-NEM labeled Coomassie blue stained proteins shown in panels aN and bW are shown in panels cN and dN respectively. Several of the proteins were radioactively labeled. Thus several of the proteins in the VKOR preparation were “candidates” for harboring the warfarin binding site. The most heavily labeled proteins 1 and 2 (see arrow heads, Fig. 2) have been identified by microsequencing. Protein 1 was identified as translation elongation factor eEF-1 beta chain (24). The very acidic protein 2 had a blocked N-terminal and tryptic digestion of the protein resulted in only two protein fragments. A high confidence N-terminal sequence was obtained for the C-terminal fragment which read SLANVNIGCLI. An extensive search of protein data banks did not result in identification of the 15 kDa protein as a known protein. Since there were no noticeable differences between the patterns of 3H-NEM labeled proteins in the VKOR preparations from normal and warfarin resistant rats this may suggest that the thiol content of the warfarin binding protein of VKOR is not altered in warfarin resistant rats. Thus the experiment did not identify a thiol containing warfarin binding protein which would be altered in the warfarin resistant rats. However VKOR in the two preparations have different kinetic properties and show different sensitivity to warfarin inhibition. VKOR from normal and warfarin resistant rats exhibit the same Km value (Km = 2.8 μM) but differ markedly in Vmax values, the enzyme in normal rats having a significantly higher catalytic rate (Vmax = 16.7 nmol/mg/30 min) than the enzyme in warfarin resistant rats (Vmax = 1.78 nmol/mg/30 min) (Fig. 3, panel B). The I50 value for warfarin inhibition of the enzyme in the two preparations were 2 and 40 μM (see Fig. 3, panel A).

The mEH Component of VKOR Is Unaltered in Warfarin Resistant Rats

Previous data from our laboratory has shown that mEH constitutes one protein component of the VKOR enzyme complex and harbors the vitamin K1 2,3-epoxide binding site of the complex (16, 17). Additional data which support this conclusion have now been acquired. When the partially purified preparation of VKOR was incubated with mEH antibodies in the presence of phospholipids, a VKOR-antibody complex could be isolated with Sepharose-protein A beads which expressed VKOR activity. The Sepharose beads from incubations with the control serum had no activity. On the other hand, the beads from incubations with the mEH antibodies exhibited a specific VKOR activity of 19,200 nmol/mg/30 min which was 662-fold higher than the specific VKOR activity measured in the partially purified VKOR preparation from normal rats. Two-dimensional SDS-PAGE of proteins bound to the washed beads identified mEH and showed a protein with a molecular weight of 32 kDa. However, we could not decide whether the 32 kDa protein was the second GST component of the complex (17) or recombinant protein A (32 kDa) released from the washed Sepharose-Protein A-antibody-antigen particles when exposed to the 9 M urea containing sample buffer used for 2-D-SDS-PAGE (data not shown).

In search for molecular differences which could account for the different properties of VKOR in normal and warfarin resistant rats, we sequenced the full length cDNAs for mEH in normal and warfarin resistant rat livers. The sequences (accession #P07687) were found to be identical in all bases. Thus the mEH component of VKOR is unaltered in normal and warfarin resistant rats.
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Assembly of the VKOR Enzyme Complex in Normal and Warfarin Resistant Rats

We have previously shown that a subpopulation of dimeric GSTs are tightly associated with the ER membrane (17) and we have proposed that these GSTs interact with the ER membrane to create catalysts intended for specific metabolic activities including vitamin K1 2,3-epoxide reduction. Within this group of GST enzymes about 40% of the enzymes are inhibited by warfarin in the GST enzyme assay (17). Furthermore we have shown that the warfarin sensitive GST enzyme loose its GST activity upon assembly of the warfarin sensitive GST activity associated with each fraction.

The dimeric GST involved in reconstitution of VKOR activity has sub-units of 32 and 29 kDa (17). If a warfarin sensitive GST enzyme is involved in forming VKOR, we expected to find a difference in warfarin sensitivity between the ER membrane associated GSTs in normal and warfarin resistant rats. Therefore we isolated from normal and warfarin resistant rats, the membrane associated dimeric GSTs and determined their sensitivity to warfarin. The purified fraction of membrane associated GSTs from normal and warfarin resistant rats are shown in Fig. 4A, lanes N and W respectively. We found the typical Ya, Yb and Yc subunit band pattern of dimeric GSTs (25) to be present in both preparations. However, as shown in Fig. 4B, the two preparations behaved differently with respect to warfarin inhibition of GST activity. The I_{50} values for warfarin inhibition of the warfarin sensitive enzymes in the preparations from normal and warfarin resistant rats were 0.7 mM and 8 mM respectively. We investigated the fate of these warfarin sensitive GST enzymes in normal and warfarin resistant rats when VKOR was assembled. For these experiments, total GST, warfarin sensitive GST and VKOR activities were determined before and after assembly of the complex by metal induced lipid aggregation as described recently (17). The data are summarized in Table 1. Addition of 10 mM MnCl₂ to detergent extracted microsomes resulted in a 40% decrease in GST activity from 79 to 48 nmol/min/mg and the remaining activity was not inhibited by warfarin. On the other hand, MnCl₂ addition was far less effective in reconstituting VKOR activity in extracted microsomes from warfarin resistant rats (2.2-fold, see Table 1). Several control experiments were carried out. These experiments showed that Mn⁺⁺ had no effect on: 1) warfarin sensitive GST activity expressed by the affinity purified GSTs, 2) this activity when the enzymes were added to extracted microsomes previously aggregated with Mn⁺⁺ and 3) the activity of another ER membrane associated enzyme, cytochrome P-450 reductase. The data show that loss of warfarin sensitive GST activity coincide with formation of the VKOR enzyme complex.

Table 1  In vitro reconstitution of VKOR from normal and warfarin resistant rats

<table>
<thead>
<tr>
<th>Fractions</th>
<th>GST Activity -Mn⁺⁺</th>
<th>+Mn⁺⁺</th>
<th>VKOR Activity -Mn⁺⁺</th>
<th>+Mn⁺⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes from normal rats</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>- warfarin</td>
<td>79 ± 1</td>
<td>47 ± 2</td>
<td>209 ± 6</td>
<td>870 ± 10</td>
</tr>
<tr>
<td>+ warfarin</td>
<td>49 ± 1</td>
<td>46 ± 2</td>
<td>12.5 ± 0.6</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Microsomes from resistant rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- warfarin</td>
<td>117 ± 5</td>
<td>101 ± 4</td>
<td>212 ± 7</td>
<td>475 ± 15</td>
</tr>
<tr>
<td>+ warfarin</td>
<td>103 ± 4</td>
<td>100 ± 3</td>
<td>185 ± 6</td>
<td>190 ± 4</td>
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Detergent extracted microsomal membranes were resuspended in buffer A for determination of GST and VKOR activity in the presence and absence of 10 mM MnCl₂. GST and VKOR activities were measured as described in Materials and Methods. Each number is the average of 3 parallel incubations ± SD. Incubations with resuspended membranes from normal and warfarin resistant rats had the same protein concentrations (2 mg/ml).

Fig. 4  Difference in warfarin inhibition of membrane associated GSTs present in normal and warfarin resistant rats. GSTs from detergent extracted microsomal membranes were isolated by agarose-GSH affinity chromatography as described in Materials and Methods. Panel A shows the Coomassie blue stained GST subunits (Ya, Yb and Yc) present in the preparations from normal rats (N) and warfarin resistant rats (W). Panel B shows warfarin inhibition of the warfarin sensitive GST activity associated with each fraction.

Fig. 5  Antibodies attached to mEH prevents the warfarin sensitive GST to become a part of the VKOR enzyme complex. The VKOR enzyme complex was assembled by metal in the presence of buffer, control serum and mEH antisemum respectively as described in Materials and Methods. GST activity was measured in each sample after Mn⁺⁺ addition. Black and open bars represent GST activity measured in the absence and presence of 5 mM warfarin. SDs are shown (n = 3)
Antibodies Attached to mEH Prevents the Warfarin Sensitive GST Enzyme to Become a Part of the VKOR Enzyme Complex

Our model of VKOR predicts that a warfarin sensitive GST enzyme “docks” with mEH in the ER membrane to form the VKOR enzyme complex (17). A stringent test of this model would be to prevent mEH from forming the complex with GST. The experiment was carried out by “coating” the mEH enzymes present in extracted microsomal membranes with mEH antibodies prior to assembling the VKOR enzyme complex as described in Materials and Methods. As shown in Fig. 5, no warfarin sensitive GST enzyme activity could be measured in the samples containing buffer and control serum (controls) following assembly of the complex by MnCl₂ addition. However, and importantly, in the samples containing the mEH antiserum, significantly more GST activity could be measured after MnCl₂ addition and this activity could be inhibited 33% by warfarin. The experiment demonstrates that the mEH antibodies “coated” the mEH enzyme and prevented its association with the warfarin sensitive GST enzyme. Thus the GST enzyme was left free to carry out its glutathione-S-transferase activity. The specificity of the mEH polyclonal antibodies was tested by comparing Coomassie blue staining and Western blots of PVDF membranes with proteins from the partially purified VKOR preparations, extracted microsomal membranes, immune complexes isolated from these mEH containing preparations and purified mEH after one-dimensional and two-dimensional SDS-PAGE of the proteins. These experiments confirmed the specificity of the antiserum.

Discussion

Warfarin is the most used anticoagulant in the world for long-term prophylaxis against thromboembolic disease (8) and has been used extensively as a poison in rodent pest control (26). The drug was introduced into clinical medicine in the mid 1950s (27) but the molecular mechanism by which the drug prevents vitamin K reduction in liver has never been unveiled. This is due to a lack of understanding of the VKOR enzyme of the vitamin K cycle which is the target for the drug (3). Indeed purification of VKOR has presented a challenge to researchers in the vitamin K field. This is now appreciated after VKOR has been identified as a lipid-protein enzyme complex in the endoplasmic reticulum membrane which easily is disrupted by chromatographic manipulations (16, 17). We have previously demonstrated that expression of VKOR activity is dependent upon association of two different enzymes; 1) mEH, an abundant liver enzyme known to be involved in detoxification of a variety of epoxides by hydroxylation (28) and 2) a warfarin sensitive ER membrane associated member of the GST gene family (25). The demonstration in this paper that the GST component of the complex in warfarin resistant rats is 10-fold less sensitive to the drug then this GST component in normal rats supports this model of VKOR.

As shown in Figs. 3 and 4, there is a large difference in the concentrations of warfarin needed to inhibit the GST activity (I₅₀ = 0.7 mM) of the free GST enzyme component of VKOR and VKOR activity (I₅₀ = 2 µM). The reason for this difference is not understood but could be the result of structural alterations of the GST enzyme upon assembly of the VKOR lipid-enzyme complex which could lead to a warfarin binding site with stronger control of enzyme activity upon drug binding.

Our data on mEH from rat liver show that mEH is not an altered protein in warfarin resistant rats. Since mEH has been shown to be a multifunctional protein [hydrolase (28), bile acid transporter (29) and a component of VKOR (16, 17)], it is reasonable to expect that the mutations causing warfarin resistance would not be found in this protein but in the warfarin sensitive GST enzyme which constitute the second component of VKOR. This hypothesis is supported by the data which show a 10-fold difference in warfarin sensitivity between the GST enzymes in normal and warfarin resistant rats. Various genotypes of warfarin resistant rats have been shown to exist (30). A future challenge will be to establish if warfarin resistance among these genotypic rats is associated with the GST component of VKOR.

The warfarin sensitive GST enzyme involved in VKOR formation is not unique among the GST family members in becoming parts of multicomponent enzyme systems. In the presence of Ca²⁺, the GST related membrane protein 5-lipoxygenase activating protein FLAP “docks” with cytosolic 5-lipoxygenase on the nuclear envelope membrane to form an enzyme complex which produces leukotriene A₄ (31). The warfarin sensitive GST enzyme may combine with a second protein in the ER membrane in a way FLAP forms a complex with 5-lipoxygenase. However FLAP is an 18 kDa integral protein of the membrane and thus is different from the dimeric membrane associated GST known from our studies to be involved in formation of VKOR (17). It is currently unknown how this dimeric GST is associated with the ER membrane (32).

Previous data have indicated that the warfarin sensitive GST enzyme involved in VKOR formation is a minor protein in rat liver (17). The enzyme was purified 6,340-fold from a detergent extract of the ER membrane. This suggests that only a small pool of the total mEH in liver is dedicated to formation of VKOR and that the GST enzyme is the limiting factor which determines how much of the complex will be made.

The γ-carboxylase is a minor protein in the ER (33). Since the γ-carboxylase and VKOR constitute an integrated γ-carboxylation system (see Fig. 1), it is reasonable to assume that VKOR and the γ-carboxylase are present at similar concentrations in the ER membrane.

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References


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