peptidyl-glutamate 4-carboxylase 4.1.1.90

1 Nomenclature

EC number
4.1.1.90

Systematic name
peptidyl-glutamate 4-carboxylase (2-methyl-3-phytyl-1,4-naphthoquinone-epoxidizing)

Recommended name
peptidyl-glutamate 4-carboxylase

Synonyms
Ci-GGC <8> [24]
GGCX <1,2,4> [21,22,23,29,30,31,32,33,34]
VKD carboxylase <2> [3,5]
carboxylase <4> [20]
γ glutamyl carboxylase <2> [23]
γ-carboxylase <1> [11]
γ-glutamyl carboxylase <1,2,4,5,6,7,8,9> (<4> polytopic membrane protein [12]) [2,7,9,11,12,13,14,15,16,17,18,19,20,21,22,24,26,27,29,30,32,33,34]
glutamate carboxylase <2,4,5,6,7,9> [1,2,5,6,7,8,9,10,13,14,15,17,18,19,20,23,25,26,27,28]
matrix Gla protein <4,5> [26]
matrix γ-carboxyglutamate protein <4,5> [26]
peptidyl-glutamate 4-carboxylase <3> [4]
peptidyl-glutamate 4-carboxylase (2-methyl-3-phytyl-1,4-naphthoquinone-epoxidizing) <3> [4]
two-chain carboxylase <2> (<2> carboxylase and epoxidase activities similar to those of one-chain carboxylase [9]) [9]
vitamin K-dependent carboxylase <2,5> [3,25]
vitamin K-dependent γ-glutamyl carboxylase <2> [31]

CAS registry number
81181-72-8

2 Source Organism

<1> *Mus musculus* [11,32]
<2> *Homo sapiens* [3,5,9,22,23,29,30,31,33,34]
<3> *Rattus norvegicus* [4]

D. Schomburg and I. Schomburg (eds.), *Class 3.4–6 Hydrolases, Lyases, Isomerases, Ligases: EC 3.4–6*, Springer Handbook of Enzymes S10, DOI 10.1007/978-3-642-36260-6_32,

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<4> *Homo sapiens* (UNIPROT accession number: P38435) [12,20,21,26,27]
<5> *Bos taurus* (UNIPROT accession number: Q07175) [1,2,8,15,16,17,18,25,26,28]
<6> *Rattus norvegicus* (UNIPROT accession number: O88496) (CTPZ [7,14]) [6,7,10,14]
<7> *Drosophila melanogaster* (UNIPROT accession number: Q9W0C4) [19]
<8> *Ciona intestinalis* (UNIPROT accession number: Q008V9) [24]
<9> *Conus textile* (UNIPROT accession number: Q8IA33) [13]

3 Reaction and Specificity

Catalyzed reaction

peptidyl-4-carboxyglutamate + 2,3-epoxyphylloquinone + H_2O = peptidyl-glutamate + CO_2 + O_2 + phylloquinone (<3> in the physiological process reaction runs in reversed direction [4])

Reaction type

carboxylation
γ-carboxylation

Natural substrates and products

S L-glutamate + CO_2 + O_2 + vitamin K hydroquinone <5,6> (Reversibility: ?) [1,2,14,28]
P γ-carboxy L-glutamate + vitamin K epoxide + H_2O
S L-glutamate + CO_2 + O_2 + vitamin K hydroquinone <5> (Reversibility: ?) [17]
P γ-carboxy-L-glutamate + vitamin K epoxide + H_2O
S peptidyl-L-glutamate + CO_2 + O_2 + menaquinone <3> (Reversibility: ?) [4]
P ?
S peptidyl-L-glutamate + CO_2 + O_2 + phylloquinone <3> (Reversibility: ?) [4]
P peptidyl-4-carboxy L-glutamate + 2,3-epoxyphylloquinone + H_2O
S Additional information <1,2,3,4,5,7> (<1> an essential posttranslational modification required for the biological activity of a number of proteins, including proteins involved in blood coagulation and its regulation [11]; <3> cis-isomer of vitamin K_1, the 2-desmethyl derivative of phylloquinone, MK-1, or menadione (2 -methyl-1,4-naphthoquinone) have little or no activity [4]; <4> enzyme accomplishes the post-translational modification required for the activity of all of the vitamin K-dependent proteins [27]; <5> enzyme catalyzes the posttranslational modification of specific glutamic acid residues to form γ-carboxygutamic acid residues within the vitamin K-dependent proteins [1]; <4> enzyme important for γ-carboxylation of gla-proteins [21]; <5> enzyme required for the posttranslational modification of vitamin K-dependent proteins [18]; <7> one of the most distinctive of the extracellular post-translational modifications is the vitamin K-dependent γ-carboxylation of glutamate residues to give γ-carboxyglutamate [19]; <2> uses the oxygenation of vitamin K to convert glutamyl residues to γ-carboxylated glutamyl residues in vitamin K-dependent

proteins [5]; <4,5> vitamin K-dependent carboxylation of glutamate to form γ-carboxyglutamate (Gla) is unusual among known posttranslational modifications in that substrate recognition does not require a specific sequence around the glutamate residues to be modified [26]; <2> vitamin K-dependent proteins require carboxylation for activity [23]) [1,4,5,11,18,19,21,23,26,27]

P ?

Substrates and products

S CDADWVEGYSMEYLSR + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> CDADWVEGYSMEYLSR [18]) (Reversibility: ?) [18]

P ? + vitamin K epoxide + H_2O

S CGRPSLEQLAQEVTYA + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> CGRPSLEQLAQEVTYA [18]) (Reversibility: ?) [18]

P ? + vitamin K epoxide + H_2O

S FLEEL + CO_2 + O_2 + ammonium sulfate <4> (<4> carboxylase activity is measured by $^{14}CO_2$ incorporation into the synthetic peptide substrate FLEEL [27]) (Reversibility: ?) [27]

P ? + vitamin K epoxide + H_2O

S FLEEL + CO_2 + O_2 + phylloquinone <5> (Reversibility: ?) [16]

P ? + 2,3-epoxyphylloquinone + H_2O

S FLEEL + CO_2 + O_2 + proFIX 19 <4> (<4> carboxylase activity is measured by $^{14}CO_2$ incorporation into the synthetic peptide substrate FLEEL [27]) (Reversibility: ?) [27]

P ? + vitamin K epoxide + H_2O

S FLEEL + CO_2 + O_2 + vitamin K hydroquinone <2,4,5,7> (<2> pentapeptide substrate FLEEL: Phe-Leu-Glu-Glu-Leu, used for carboxylation activity [9]) (Reversibility: ?) [1,9,19,20]

P ? + vitamin K epoxide + H_2O

S FLEEL + CO_2 + O_2 + vitamin K_1 hydroquinone <1> (<1> a synthetic peptide substrate, assay for vitamin K-dependent carboxylase activity [11]) (Reversibility: ?) [11]

P ? + vitamin K_1 epoxide + H_2O

S FLEEL + CO_2 + O_2 + vitamin KH_2 <4,5,9> (<4> carboxylase activity is measured by $^{14}CO_2$ incorporation into the synthetic peptide substrate FLEEL [27]; <5> Phe-Leu-Glu-Glu-Leu [25]) (Reversibility: ?) [2,8,13,17,25,27]

P ? + vitamin K epoxide + H_2O

S FLEEV + CO_2 + O_2 + vitamin K hydroquinone <5> (Reversibility: ?) [15]

P ? + vitamin K epoxide + H_2O

S GKDRLTQMKRILKQRGNKARGEEELY + CO_2 + O_2 + vitamin K hydroquinone <7> (Reversibility: ?) [19]

P ? + vitamin K epoxide + H_2O

S L-glutamate + CO_2 + O_2 + vitamin K hydroquinone <2,4,5> (<5> catalyzes modification of specific glutamic acids to γ-carboxyglutamic acid in several blood-coagulation proteins [25]; <4> reaction is essential for the

activity of all of the vitamin K-dependent proteins [27]) (Reversibility: ?) [17,23,25,27]
P γ-carboxy-L-glutamate + vitamin K epoxide + H_2O
S L-glutamate + CO_2 + O_2 + vitamin K hydroquinone <1,2,4,5,6,7,9> (<5> vitamin K epoxide must be recycled to vitamin K hydroquinone by the enzyme epoxide reductase for the reaction to continue [18]) (Reversibility: ?) [1,2,3,7,11,12,13,14,18,19,20,26,28]
P γ-carboxy L-glutamate + vitamin K epoxide + H_2O
S N-(bromoacetyl)-FLEELY + CO_2 + O_2 + vitamin KH_2 <5> (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S ProFIX 19-6BPA + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> TVFLDHENANKIBNRPKR [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S ProFIX 19-7BPA + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> TVFLDHENfiNKBLNRPKR [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S ProFIX19-13BPA + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> TVFLDBENWKILNRPKRY [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S ProFIX19-16BPA + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> TVBLDHENANKILNRPKRY [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S TVFLDHENANKILNRPKRANTBLEEVRK + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> carboxylase probe1, TVFLDHENANKILNRPKRANTBLEEVRK as a substrate [15]) (Reversibility: ?) [15]
P ? + vitamin K epoxide + H_2O
S TVFLDHENANKILNRPKRYNTBLEEVRK + CO_2 + O_2 + vitamin K hydroquinone <5> (Reversibility: ?) [15]
P ? + vitamin K epoxide + H_2O
S YVFLDHQDADANLILNRPKR + CO_2 + O_2 + vitamin KH_2 <5> (Reversibility: ?) [2]
P ? + vitamin K epoxide + H_2O
S conantokin G + CO_2 + O_2 + vitamin K hydroquinone <7> (<7> poorly carboxylated [19]) (Reversibility: ?) [19]
P ? + vitamin K epoxide + H_2O
S conotoxin ε-TxIX + CO_2 + O_2 + vitamin KH_2 <9> (Reversibility: ?) [13]
P ?
S e-TxIX12 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues 1-12 of e-TxIX [13]) (Reversibility: ?) [13]
P ?
S γ-carboxylated glutamyl containing vitamin K-dependent protein + vitamin K epoxide + H_2O <2> (Reversibility: ?) [5]
P ?
S glutamyl containing vitamin K-dependent protein + CO_2 + vitamin K hydroquinone + O_2 <2> (<2> propeptide binding increases carboxylase affinity for the Glu substrate, and the coordinated binding of the vitamin K-

dependent propeptide and Glu substrate increase carboxylase affinity for vitamin K and activity, possibly through a mechanism of substrate-assisted catalysis. The propeptide adjacent to the Gla domain is cleaved subsequently to carboxylation. The carboxylase uses the energy of vitamin K hydroquinone oxygenation to convert glutamyl residues to γ-carboxylated glutamyl residues in vitamin K-dependent proteins. During carboxylation, the vitamin K hydroquinone cofactor is oxidized to a vitamin K epoxide product. The carboxylase itself is also a vitamin K-dependent protein and carboxylase carboxylation may be important in regulating the overall process of vitamin K-dependent protein carboxylation. All vitamin K-dependent proteins contain multiple glutamyl residues that undergo carboxylation, which is accomplished by a processive mechanism. A single binding event between carboxylase and vitamin K-dependent protein can give rise to all of the glutamyl to gamm-carboxylated glutamyl conversions in the vitamin K-dependent protein. Carboxylation is limited to the glutamyl residue residing within the Gla domain substrate [5]) (Reversibility: ?) [5]

P ?

S osteocalcin + reduced vitamin K + CO_2 + O_2 <6> (Reversibility: ?) [7]

P ? + vitamin K epoxide + H_2O

S *p*-benzoylphenylalanine + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> BPA [18]) (Reversibility: ?) [18]

P ? + vitamin K epoxide + H_2O

S peptidyl-L-glutamate + CO_2 + O_2 + menaquinone <3> (Reversibility: ?) [4]

P ?

S peptidyl-L-glutamate + CO_2 + O_2 + phylloquinone <3> (Reversibility: ?) [4]

P peptidyl-4-carboxy L-glutamate + 2,3-epoxyphylloquinone + H_2O

S pro-FIX19 + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> pro-FIX19: peptide comprising residues of human factor IX AVFLDHENANKILNRPKRY [18]) (Reversibility: ?) [18]

P ? + vitamin K epoxide + H_2O

S pro-FIX19-16BPA + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> pro-FIX19-16BPA: peptide comprising residues TVBLDHENANKILNRPKRY [18]) (Reversibility: ?) [18]

P ? + vitamin K epoxide + H_2O

S pro-e-TxIX/12 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -12 to -1 of e-TxIX precursor [13]) (Reversibility: ?) [13]

P ?

S pro-e-TxIX/24 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -12 to +12 of e-TxIX precursor [13]) (Reversibility: ?) [13]

P ?

S pro-e-TxIX/41 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -29 to +12 of e-TxIX precursor [13]) (Reversibility: ?) [13]

P ?

S proFIX 19 + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> proFIX 19, peptide sequence: TVFLDHENANKILNRPKRY [15]) [15]

P ? + vitamin K epoxide + H_2O
S proFIX/PT28 + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> proFIX/PT28, peptide sequence: TVFLDHENANKILNRPKRANTFLEEVRK [15]) (Reversibility: ?) [15]
P ? + vitamin K epoxide + H_2O
S proFIX18 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -18 to -1 of proFactor IX [13]) (Reversibility: ?) [13]
P ?
S proFIX19 + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> TVFLDHENANKILNRPKRY [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S proFIX28 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -18 to +10 of proFactor IX [13]) (Reversibility: ?) [13]
P ?
S proFIXl9 + CO_2 + O_2 + vitamin KH_2 <5> (<5> AVFLDHENANKILNRPKRY [25]) (Reversibility: ?) [25]
P ? + vitamin K epoxide + H_2O
S proPT18 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -18 to -1 of prothrombin. 28-residue peptides based on residues -18 to +10 of human proprothrombin and proFactor IX with K_m values of 420 lM, 1.7 μM and 6ÎmicroM [13]) (Reversibility: ?) [13]
P ?
S proPT28 + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> HVFLAPQQARSLLQRVRRANTFLEEVRK [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S proPT28 + CO_2 + O_2 + vitamin K hydroquinone <5> (<5> proPT28: synthetic peptide is designated by the following nomenclature: pro indicates the presence of the propeptide sequence, PT indicates prothrombin, the protein on which the peptide sequence is based, and 28 indicates the number of amino acid residues in the peptide [1]) (Reversibility: ?) [1]
P ? + vitamin K epoxide + H_2O
S proPT28 + CO_2 + O_2 + vitamin KH_2 <9> (<9> residues -18 to +10 of proprothrombin [13]) (Reversibility: ?) [13]
P ?
S proPTl8 + CO_2 + O_2 + FLEEL + vitamin KH_2 <5> (<5> HVFLAPQQARSLLQRVRR [8]) (Reversibility: ?) [8]
P ? + vitamin K epoxide + H_2O
S Additional information <1,2,3,4,5,6,7,9> (<1> an essential posttranslational modification required for the biological activity of a number of proteins, including proteins involved in blood coagulation and its regulation [11]; <3> cis-isomer of vitamin K_1, the 2-desmethyl derivative of phylloquinone, MK-1, or menadione (2 -methyl-1,4-naphthoquinone) have little or no activity [4]; <4> enzyme accomplishes the post-translational modification required for the activity of all of the vitamin K-dependent proteins [27]; <5> enzyme catalyzes the posttranslational modification of specific glutamic acid residues to form γ-carboxygutamic acid residues within the vitamin K-dependent proteins [1]; <4> enzyme important for γ-car-

boxylation of gla-proteins [21]; <5> enzyme required for the posttranslational modification of vitamin K-dependent proteins [18]; <7> one of the most distinctive of the extracellular post-translational modifications is the vitamin K-dependent γ-carboxylation of glutamate residues to give γ-carboxyglutamate [19]; <2> uses the oxygenation of vitamin K to convert glutamyl residues to γ-carboxylated glutamyl residues in vitamin K-dependent proteins [5]; <4,5> vitamin K-dependent carboxylation of glutamate to form γ-carboxyglutamate (Gla) is unusual among known posttranslational modifications in that substrate recognition does not require a specific sequence around the glutamate residues to be modified [26]; <2> vitamin K-dependent proteins require carboxylation for activity [23]; <9> amino-acid sequences of the synthetic substrates and propeptides are shown [13]; <4,5> identification of a striking homology between exon 3 in all known matrix Gla proteins and a 24-residue sequence in the bovine and human γ-glutamyl carboxylases. Alignment of exon 3 of matrix Gla protein with the homologous region of the γ-glutamyl carboxylase shown [26]; <5> photolabeling of Q-glutamyl carboxylase with Bpa peptides [15]; <6> separate active sites are required to support vitamin K-dependent epoxide formation and carboxylation. The binding site for vitamin K oxygenase contains an active thiol group [6]; <5> vitamin K carboxylase specifically interacts with the propeptide region of the precursor forms of vitamin K dependent proteins [8]) [1,4,5,6,8,11,13,15,18,19,21,23,26,27]

P ?

Inhibitors

2,3,5,6-tetrachloro-4-pyridinol <3> (<3> TCP, anticoagulant action, effective in vitro inhibitor of the carboxylase [4]) [4]

2-chloro-3-phytyl-1,4-naphthoquinone <3,5> (<3> chloro-K, effective in vivo antagonist of vitamin K, inhibits the enzyme in an competitive fashion [4]) [4,28]

Boc-(2S,4S)-4-methylglutamic acid-Glu-Val <4> (<4> competitive inhibitor, FLEEL as substrate [20]) [20]

Boc-Ser(OPO_4)-Ser(OPO_4)-Leu-OMe <3> (<3> inhibits the enzyme apparently competitively with regard to other peptide substrate [4]) [4]

CN^- <3> (<3> enzyme is blocked by mM concentrations of CN^- [4]) [4]

CsCl <6> [10]

Cu^{2+} <3> (<3> free Cu^{2+} and Cu^{2+}-complexes inhibit the reaction [4]) [4]

FFRCK <5> (<5> peptide, protease inhibitor [25]) [25]

FPRCK <5> (<5> peptide, protease inhibitor [25]) [25]

N-ethylmaleimide <5,6> (<6> preincubation with vitamin K hydroquinone prevents NEM inhibition of epoxide formation but not of carboxylation [6]) [6,16]

TVFLDHENANKILNRPKRANTBLEEVRK <5> (<5> the enzyme is photoirradiated on ice at 365 nm with TVFLDHENANKILNRPKRANTBLEEVRK, TVFLDHENANKILNRPKRYNTBLEEVRK and mono [127I]TVFLDHENANKILNRPKRYNTBLEEVRK for various times [15]) [15]

TVFLDHENANKILNRPKRYNTBLEEVRK <5> (<5> the enzyme is photoirradiated on ice at 365 nm with TVFLDHENANKILNRPKRANTBLEEVRK, TVFLDHENANKILNRPKRYNTBLEEVRK and mono [127I]TVFLDHENAN KILNRPKRYNTBLEEVRK for various times. Presence of TVFLDHENAN KILNRPKRYNTBLEEVRK or its iodinated derivative 80% inactivation is observed [15]) [15]
anti-carboxylase antiserum <5> (<5> effect of anti-carboxylase antiserum on carboxylase activity: under the conditions employed the carboxylation is inhibited by 80%, with parallel inhibition of CO_2 incorporation into FLEEL and proPT28 (synthetic peptide) [1]) [1]
bromoacetyl-FLEEL peptide <5> (<5> the His6-carboxylase is irreversibly inactivated. Up to 85% of the carboxylase activity is lost over a period of 120 min [17]) [17]
deoxycholate <6> [10]
ethanol <3> (<3> high concentrations [4]) [4]
hematin <3> [4]
iodoacetic acid <6> (<6> poor inhibitor [6]) [6]
methemoglobin <3> [4]
p-chloromercuribenzoate <6> (<6> 97% inhibition with 1.25 mM and at 5 mM the reaction is completely inhibited [10]) [10]
p-hydroxymercuribenzoate <3,6> (<6> 1 mM, more than 90% inhibition, inhibition is reversed by dithiothreitol [6]) [4,6]
proFIX/PT28 (Bpa +4) <5> (<5> presence of proFIX/PT28 (Bpa +4) or its iodinated derivative 56% inactivation is observed [15]) [15]
proFIX19-16BPA propeptide <5> [8]
protease inhibitor mixture <5> (<5> PIC, freshly prepared as a 10x PIC stock containing 20 mM dithiothreitol, 20 mM EDTA, FFRCK (1.25 μg/ml), FPRCK (1.25 μg/ml), leupeptin (5 μg/ml), pepstatin A (7 μg/ml), phenylmethylsulfonyl fluoride (340 μg/ml), aprotinin (20 μg/ml) [25]) [25]
sucrose <6> [10]
tetrachloropyridin <5> [28]
trypsin <6> [10]
vitamin K <5> (<5> carboxylation of FLEEL by bovine liver carboxylase is inhibited by high concentrations of vitamin KH_2. vitamin K (up to 400 mM) and vitamin K epoxide (up to 1 mM) are not inhibitory. R234A/H235A mutant, R406A/H408A mutant, and R513A/K515A mutant are more susceptible to inhibition by vitamin KH_2 than wild type enzyme. R234A/H235A mutant and R406A/H408A mutant exhibit maximal activity at 111 mM vitamin KH_2 and R513A/K515A mutant at 56 mM vitamin KH_2 [2]) [2]
warfarin <2,5,6> [10,16,23]
Additional information <2,5,6> (<5> 15 min irradiation in the absence of peptide resulted in a 10% inactivation of the carboxylase [15]; <5> in the presence of high concentrations of propeptide, only minimal carboxylase activity is measurable. Antibodies to the protein inhibit the carboxylase activity in crude preparations [1]; <6> NADH, dithiothreitol, and ATP deficiency decrease enzyme activity [10]; <2> the carboxylase reaction is inhibited by sulfhydryl-specific reagents [23]) [1,10,15,23]

Cofactors/prosthetic groups

phylloquinone <5> [16]

vitamin K <2,3,4,5,6,7,8> (<6> aquamephyton [10]; <4> carboxylase and soybean seed lipoxygenase share 19.3% identity over a span of 198 amino acids, from residues 468 to 666 of carboxylase. This is interesting because the carboxylase acts as an oxygenase on the cofactor vitamin K-hydroquinone, and the similarity occurs in that region of the carboxylase likely to have enzymatic function [27]; <4> conversion of glutamic acid to γ-carboxyglutamic acid is coupled with the oxygenation of KH_2 to vitamin K 2,3-epoxide and has been referred to as vitamin K epoxidase activity [20]; <7> phytonadione [19]; <2> the carboxylase uses the energy of vitamin K hydroquinone oxygenation to convert glutamyl residues to γ-carboxylated glutamyl residues in vitamin K-dependent proteins. During carboxylation, the vitamin K hydroquinone cofactor is oxidized to a vitamin K epoxide product [5]) [1,2,3,4,5,6,7,8,9,10,12,14,15,17,18,19,20,21,23,24,25,26,27,28]

vitamin K_1 <1,9> [11,13]

Activating compounds

ammonium sulfate <4> [27]

benzoylphenylalanine <5> (<5> Bpa, the four Bpa peptides enhance γ-carboxylation by 1.5-2.3fold, and the rate enhancement profiles are very similar to that of proFIX19, showing that these propeptides are recognized by the carboxylase [8]) [8]

decarboxylated plasma prothrombin <3> (<3> activates the enzyme [4]) [4]

endogenous precursor <3> (<3> activates the enzyme [4]) [4]

proFIX 19 <4> [27]

proFIX19 <5> (<5> enhances γ-carboxylation of the synthetic FLEEL peptide by 2.2-2.3fold [8]) [8]

proPT18 <5> (<5> enhances γ-carboxylation of the synthetic FLEEL peptide by 2.2-2.3fold [8]) [8]

Additional information <3> (<3> enzyme activity increases 2-3fold by a vitamin K deficiency or Warfarin treatment [4]) [4]

Metals, ions

Additional information <3,5> (<5> calcium-binding protein [1]; <3> Mn^{2+}, high concentrations significantly decrease or have no effect on K_m of a peptide substrate for the enzyme [4]) [1,4]

Turnover number (s^{-1})

0.0019 <4> (FLEEL, <4> Y395A, pH 7.4, 20°C [20]) [20]

0.002 <5> (YVFLDHQDADANLILNRPKR, <5> R359A/H360A/K361A mutant [2]) [2]

0.0033 <4> (FLEEL, <4> W399A, pH 7.4, 20°C [20]) [20]

0.01 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0 μM [2]) [2]

0.015 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0 μM [2]) [2]

0.018 <5> (vitamin KH_2, <5> proPT28, R513A/K515A mutant [2]) [2]

0.02 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.02 <5> (vitamin KH_2, <5> proPT28, FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.025 <5> (vitamin KH_2, <5> proPT28, R406A/H408A mutant [2]) [2]
0.033 <5> (vitamin KH_2, <5> proPT28, R234A/H235A mutant [2]) [2]
0.042 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant [2]) [2]
0.056 <5> (YVFLDHQDADANLILNRPKR, <5> R513A/K515A mutant [2]) [2]
0.059 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 0 μM [2]) [2]
0.06 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant [2]) [2]
0.07 <5> (FLEEL, <5> R513A/K515A mutant [2]) [2]
0.1 <5> (vitamin KH_2, <5> FLEEL, R513A/K515A mutant [2]) [2]
0.11 <4> (FLEEL, <4> L394R, pH 7.4, 20°C [20]) [20]
0.11 <5> (vitamin KH_2, <5> FLEEL, R359A/H360A/K361A mutant [2]) [2]
0.153 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0.16 μM [2]) [2]
0.175 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0.16 μM [2]) [2]
0.19 <5> (FLEEL, <5> R359A/H360A/K361A mutant [2]) [2]
0.21 <4> (FLEEL, <4> wild type, pH 7.4, 20°C [20]) [20]
0.324 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 0.16 μM [2]) [2]
0.347 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 1.6 μM [2]) [2]
0.391 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 1.6 μM [2]) [2]
0.465 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 1.6 μM [2]) [2]
0.488 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 160 μM [2]) [2]
0.56 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 160 μM [2]) [2]
0.56 <5> (vitamin KH_2, <5> FLEEL, R406A/H408A mutant [2]) [2]
0.6 <5> (FLEEL, <5> for wild type FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.6 <5> (vitamin KH_2, <5> FLEEL, R234A/H235A mutant [2]) [2]
0.64 <5> (FLEEL, <5> R234A/H235A mutant [2]) [2]

0.645 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 160 microM [2]) [2]
0.66 <5> (vitamin KH_2, <5> FLEEL, FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.7 <5> (FLEEL, <5> wild type [2]; <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.72 <5> (FLEEL, <5> R406A/H408A mutant [2]) [2]
1 <5> (CO_2, <5> pH 7.4 [16]) [16]
1 <5> (FLEEL, <5> wild-type carboxylase in bovine liver microsomes [17]) [17]
1.7 <5> (FLEEL, <5> recombinant His6-carboxylase present in Sf9 cell microsomes [17]) [17]
Additional information <4> (<4> k_{cat} values relative to wild type are 51% (L394R), 1% (Y395A), and 2% (W399A), pH 7.4, 20°C [20]) [20]

Specific activity (U/mg)

0.0000026 <2> (<2> H160A mutant [3]) [3]
0.0000027 <2> (<2> wild type [3]) [3]
0.0000045 <2> (<2> H381A mutant [3]) [3]
Additional information <5> (<5> propeptide eluate of gel: 608 U/mg, carboxylase units are expressed as 100000 dpm of $^{14}CO_2$ fixed in the standard assay [28]; <5> specific activities of the partially purified microsomes and the homogeneous vitamin K-dependent carboxylase are 2.77 and 279.6 nmol of vitamin K epoxide per h per mg of protein, a 101fold purification of the vitamin K epoxidase activity from partially purified microsomes [1]) [1,28]

K_m-Value (mM)

0.0017 <9> (28-residue peptides based on residues -18 to +10 of human proprothrombin, <9> pH 7.0 [13]) [13]
0.0023 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.0038 <5> (vitamin KH_2, <5> proPT28, R406A/H408A mutant [2]) [2]
0.004 <5> (vitamin KH_2, <5> proPT28, FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.0041 <5> (YVFLDHQDADANLILNRPKR, <5> R359A/H360A/K361A mutant [2]) [2]
0.0052 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant [2]) [2]
0.006 <9> (proFactor IX, <9> pH 7.0 [13]) [13]
0.0068 <5> (TVFLDHENANKILNRPKRANTBLEEVRK, <5> carboxylase probe1, TVFLDHENANKILNRPKRANTBLEEVRK as a substrate [15]) [15]
0.007 <5> (vitamin KH_2, <5> proPT28, R234A/H235A mutant [2]) [2]
0.0081 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant [2]) [2]
0.0123 <5> (vitamin KH_2, <5> proPT28, R513A/K515A mutant [2]) [2]
0.0144 <5> (YVFLDHQDADANLILNRPKR, <5> R513A/K515A mutant [2]) [2]

0.032 <5> (vitamin KH_2, <5> FLEEL, R513A/K515A mutant [2]) [2]
0.043 <5> (vitamin KH_2, <5> FLEEL, R359A/H360A/K361A mutant [2]) [2]
0.052 <9> (vitamin K_1, <9> pH 7.0 [13]) [13]
0.053 <5> (vitamin KH_2, <5> FLEEL, R406A/H408A mutant [2]) [2]
0.074 <9> (precursor analog containing 29 amino acids of the propeptide region, <9> pH 7.0 [13]) [13]
0.074 <5> (vitamin KH_2, <5> FLEEL, FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
0.075 <9> (precursor analog containing 12 of the propeptide region, <9> pH 7.0 [13]) [13]
0.097 <5> (vitamin KH_2, <5> FLEEL, R234A/H235A mutant [2]) [2]
0.3 <5> (CO_2, <5> pH 7.4 [16]) [16]
0.42 <9> (FLEEL, <9> pH 7.0 [13]) [13]
0.54 <4> (FLEEL, <4> wild type, pH 7.4, 20°C [20]) [20]
0.565 <9> (conotoxin ε-TxIX, <9> pH 7.0 [13]) [13]
0.78 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 1.6 μM [2]; <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 160 μM [2]) [2]
0.91 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADAN LILNRPKR concentration 0.16 μM [2]) [2]
0.92 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 160 μM [2]) [2]
1 <5> (FLEEL, <5> wild type [2]) [2]
1.01 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, proIX18 concentration 160 μM [2]) [2]
1.1 <5> (FLEEL, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
1.23 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 1.6 μM [2]) [2]
1.25 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 1.6 μM [2]) [2]
1.3 <5> (FLEEL, <5> R234A/H235A mutant [2]) [2]
1.5 <5> (FLEEL, <5> for wild type FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]; <5> proPTl8 propeptide [8]; <5> R359A/H360A/K361A mutant [2]; <5> wild-type carboxylase in bovine liver microsomes [17]) [2,8,17]
1.54 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0.16 μM [2]) [2]
1.6 <5> (FLEEL, <5> pH 7.4 [16]; <5> proFIX19 propeptide [8]; <5> R406A/H408A mutant [2]; <5> recombinant His6-carboxylase present in Sf9 cell microsomes [17]) [2,8,16,17]

1.78 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0.16 μM [2]) [2]
1.8 <5> (FLEEL, <5> proFIX 19-6BPA propeptide [8]) [8]
1.9 <5> (FLEEL, <5> proFIX19-16BPA propeptide [8]; <5> R513A/K515A mutant [2]) [2,8]
2 <5> (FLEEL, <5> proFIX19-13BPA propeptide [8]; <5> proFIX19-7BPA-propeptide [8]) [8]
2.01 <5> (YVFLDHQDADANLILNRPKR, <5> FLAG-vitamin K-dependent γ-glutamyl carboxylase, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0 μM [2]) [2]
2.03 <5> (YVFLDHQDADANLILNRPKR, <5> R234A/H235A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0 microM [2]) [2]
2.06 <5> (YVFLDHQDADANLILNRPKR, <5> R406A/H408A mutant, peptides comprising residue YVFLDHQDADANLILNRPKR concentration 0 μM [2]) [2]
6.49 <4> (FLEEL, <4> L394R, pH 7.4, 20°C [20]) [20]
9 <5> (FLEEL, <5> none propeptide [8]) [8]
14.8 <4> (FLEEL, <4> Y395A, pH 7.4, 20°C [20]) [20]
24.3 <4> (FLEEL, <4> W399A, pH 7.4, 20°C [20]) [20]
Additional information <5> (<5> K_m-values measured by hyperbolic weighted least-squares analysis [8]) [8]

K_i-Value (mM)

0.013 <4> (Boc-(2S,4S)-4-methylglutamic acid-Glu-Val, <4> wild type, pH 7.4, 20°C [20]) [20]
0.0174 <5> (proFIX19-16BPA propeptide, <5> competitive inhibition experiments using prom28 substrate [8]) [8]
1.4 <4> (Boc-(2S,4S)-4-methylglutamic acid-Glu-Val, <4> L394R, pH 7.4, 20°C [20]) [20]
2.1 <4> (Boc-(2S,4S)-4-methylglutamic acid-Glu-Val, <4> Y395A, pH 7.4, 20°C [20]) [20]
Additional information <4> (<4> K_i value for Boc-(2S,4S)-4-methylglutamic acid-Glu-Val is above 5 mM for W399A, pH 7.4, 20°C [20]) [20]

pH-Optimum

7.2-7.4 <3> (<3> assay at. The activity falls off sharply above pH 8 or below pH 7 [4]) [4]
7.5 <2> [3]
8.5 <2> (<2> assay at [3]) [3]

pH-Range

5.5-8.5 <2> [3]

4 Enzyme Structure

Molecular weight

33000 <9> (<9> cells transfected with the CAT-V5/His plasmid, Western Blot [13]) [13]
60000 <4> (<4> proteinase K digestion of the hGC-FLAG reveals a 60 kDa fragment, indicating the lumenal location of the FLAG tag and therefore the carboxyl-terminus of the carboxylase. In contrast, FLAG-hGC does not show a proteinase K-resistant fragment except for the residual undigested full-length carboxylase, which indicates the cytoplasmic location of the FLAG tag and therefore the amino-terminus of the carboxylase [12]) [12]
77000 <5> (<5> single major band on SDS gel electrophoresis. The eluted protein contains both stable vitamin K-dependent carboxylase and vitamin K epoxidase activity [1]) [1]
85700 <6> (<6> calculated from sequence [14]) [14]
87540 <4> (<4> calculated from sequence [27]) [27]
94000 <4,5> (<4,5> SDS-PAGE [8,18,25,27]; <5> SDS-PAGE and Western Blot, His6-carboxylase-Ac-FLEEL confirming affinity-purified carboxylase [17]) [8,17,18,25,27]
95000 <4> (<4> full-length carboxylase [12]) [12]
99000 <5> (<5> SDS-PAGE, Western Blot. CHO cells transfected with the cDNA for wild type FLAG-vitamin K-dependent γ-glutamyl carboxylase [2]) [2]
130000 <9> (<9> SDS-PAGE, Sf21 cells transfected with the carboxylase cDNA-containing plasmid, Western Blot [13]) [13]
Additional information <2> (<2> determination of disulfide bond formation in purified two-chain carboxylase and P80L and P378L two-chain carboxylases by SDS-PAGE and Western Blot analyses [9]) [9]

Subunits

? <4,5> (<4,5> x * 94000, SDS-PAGE [8,18,25,27]; <5> x * 99000, SDS-PAGE [2]) [2,8,18,25,27]
monomer <5> (<5> 1 * 77000, SDS-PAGE [1]) [1,17]
Additional information <2,5> (<5> binding site in the carboxyl half of the γ-glutamyl carboxylase. Carboxylase may be cleaved by trypsin into an amino-terminal 30 kDa and a carboxyl-terminal 60 kDa fragment joined by disulfide bond(s), and the propeptide binds to the 60 kDa fragment [18]; <2> disulfide bond between cysteines 99 and 450, five transmembrane domains [23]; <2> five transmembrane domains. Transmembrane domain interactions and residue proline 378 are essential for proper structure, especially disulfide bond formation [9]; <5> limited tryptic digestion of the carboxylase yields two disulfide-linked fragments with molecular masses of 30 and 60 kDa, corresponding to the amino and carboxy-terminal part of the γ-glutamyl carboxylase [15]) [9,15,18,23]

Posttranslational modification

glycoprotein <2> [9]
Additional information <4> (<4> enzyme catalyzes vitamin K-dependent posttranslational modification of glutamate to γ-carboxyl glutamate [12]) [12]

5 Isolation/Preparation/Mutation/Application

Source/tissue

CJ7 cell <1> (<1> γ-carboxylase gene targeting [11]) [11]
H4-II$^-$E-C_3 cell <6> (<6> rat hepatoma cell line [14]) [14]
HEK-293 cell <2,4> [23,27]
R1 cell <1> (<1> γ-carboxylase gene targeting [11]) [11]
S2 cell <7> [19]
blood plasma <1> (<1> coagulation assays [11]) [11]
bone <3> [4]
cartilage <3> [4]
embryo <6> (<6> vitamin K-dependent carboxylase mRNA expression in early rat embryonic development [14]) [14]
epithelial cell <2> [34]
fat <6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [14]
fibroblast <3> [4]
heart <6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [14]
kidney <3,6> (<6> adult Sprague-Dawley rat [14]) [4,14]
kidney cell line <3> [4]
liver <1,3,5,6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]; <1> preparation of solubilized γ-carboxylase [11]) [1,2,4,6,7,8,10,11,14,15,16,17,25,28]
lung <3,6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [4,14]
muscle <6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [14]
ovary <6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [14]
pancreas <3,6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [4,14]
placenta <3> [4]
renal tubule <2> [34]
spleen <3,6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [4,14]
stomach <6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [14]
tendon <3> [4]

testis <3,6> (<6> expression of rat vitamin K-dependent carboxylase in adult and embryonic tissues [14]) [4,14]
thymus <3> [4]
thyroid <3> [4]
uterus <3> [4]
Additional information <2> (<2> 6-year-old Mexican American male [33]) [33]

Localization

cytoplasm <2,4> (<4> human glutamyl carboxylase spans the membrane at least 5times, with its N-terminus in the cytoplasm and its C-terminus in the lumen of the endoplasmic reticulum [12]) [12,34]
endoplasmic reticulum <1,2,4,5,6> (<4> rough [20]; <1> transmembrane protein [11]; <2> potential impact of quality control components on carboxylation, which occurs in the endoplasmic reticulum during the secretion of vitamin K-dependent proteins [5]) [5,7,9,11,18,20,23]
endoplasmic reticulum membrane <4,5> (<4> amino-terminus of the γ-glutamyl carboxylase is on the cytoplasmic side of the endoplasmic reticulum, while the carboxylterminus is on the lumenal side [12]) [2,12]
membrane <4,5> (<5> integral membrane protein [18]; <4> 758 residue integral membrane protein [27]) [1,18,27]
microsome <2,3,4,5,6> (<5> from a dicoumarol-treated cow [28]; <4> microsomal carboxylase activity is compared from cells transfected with pCMV5 [27]) [1,2,4,6,8,9,10,16,17,25,27,28]
rough endoplasmic reticulum <3> [4]

Purification

<2> [9]
<2> (affinity chromatography) [3]
<3> (difficult, different methods shown) [4]
<4> [20]
<5> [8,16]
<5> (affinity chromatography) [1]
<5> (degree of purification is about 7000fold with reference to ammonium sulfate-fractionated microsomal protein from liver. Purification of carboxylase, solubilized microsomes: 281000 cpm/mg/h, flow-through of Affi-FIXQ/S: 200000 cpm/mg/h, bound to Affi-FIXQ/S: 140000000 cpm/mg/h, affinity-purified carboxylase: 1930000000 cpm/mg/h) [25]
<5> (inactivated His6-carboxylase-Ac-FLEEL purified under denaturing conditions by Ni-chelation chromatography followed by preparative polyacrylamide gel electrophoresis) [17]
<5> (partial purification of the enzyme by antibody affinity techniques. Purified 500fold by adsorption to an antiprothrombin column and elution with a dodeca peptide which competes with a prothrombin precursor enzyme recognition site. The purified enzyme is devoid of bound precursors, and has the same ratio of vitamin K epoxidase activity to carboxylase activity as the crude microsomal preparation) [28]
<6> [6]

Cloning

<2> [23]
<2> (expressed in Sf9 cells) [9]
<2> (mutational analysis is performed using an expression system lacking endogenous carboxylase. Construction of baculo viruses containing FLAG-tagged carboxylase mutants and expression in infected SF21 cells. Expresses the 758 amino acid human VKD carboxylase bearing a C-terminal extension of AAADYKDDDDK, where the last eight amino acids are the FLAG epitope) [3]
<4> (expressed in 293 cells) [12]
<4> (expression of the cloned cDNA results in an increase in carboxylase activity in microsomes of transfected cells compared to mock-transfected cells) [27]
<5> (His6-tagged bovine liver carboxylase (His6-carboxylase) is produced in insect cells using a baculovirus expression system) [8]
<5> (expressed in Chinese hamster ovary cells with an immunodetectable octapeptide inserted at the amino-terminal ends) [2]
<5> (expressed in baculovirus-infected insect cell. Produced His6-tagged carboxylase as a recombinant protein using a baculovirus expression system) [17]
<6> [7,14]
<9> (expression in COS cells or expressed in Sf21 insect cells) [13]

Engineering

E373L/Q374L <2> (<2> transmembrane domain residues in the C-terminal peptide to test for polar/charge residues [9]) [9]
E567A/K569A <5> (<5> CBX567/568 [2]) [2]
E612A/D614A <5> (<5> CBX612/614 [2]) [2]
G125L <2> (<2> two-chain carboxylase [9]) [9]
G128L <2> (<2> two-chain carboxylase [9]) [9]
G132L <2> (<2> two-chain carboxylase [9]) [9]
G363L/T367L <2> (<2> transmembrane domain residues in the C-terminal peptide to test for polar/charge residues [9]) [9]
H160A <2> (<2> His to Ala mutants all show full epoxidase activity [3]) [3]
H177A/H178A <5> (<5> CBX177/178 [2]) [2]
H287A <2> (<2> His to Ala mutants all show full epoxidase activity [3]) [3]
H381A <2> (<2> His to Ala mutants all show full epoxidase activity [3]) [3]
H404A <4> (<4> carboxylases W390A, S398A, and H404A have activities similar to that of wild type [20]) [20]
H678A/E679A/R680A <5> (<5> CBX678/679/680 [2]) [2]
K217A/K218A <5> (<5> inactive, CBX217/218 [2]) [2]
K218A <2> (<2> K218A activity is not detectable. The addition of exogenous amines restores K218A activity while having little effect on wild type carboxylase [3]) [3]
K346A/R347A <5> (<5> CBX346/347 [2]) [2]
K438A/D439A/H440A <5> (<5> CBX438/439/440 [2]) [2]
K622A/E623A/K624A <5> (<5> CBX622/623/624 [2]) [2]

L128R <2> (<2> warfarin resistent mutant [23]) [23]
L368/372P <2> (<2> mutation to disrupt the transmembrane helix [9]) [9]
L394R <4> (<4> natural mutant, certain individuals with combined deficiencies of vitamin K-dependent proteins have a mutation, L394R, in their γ-glutamyl carboxylase causing impaired glutamate binding [20]) [20]
P378L <2> (<2> significantly decreases the disulfide formation in carboxylase [9]) [9]
P80L <2> (<2> mutation of residue P80, which has activity similar to that of wild-type carboxylase, has a minor effect on disulfide formation [9]) [9]
R189A/K190A/R191A <5> (<5> CBX189/190/191 [2]) [2]
R234A/H235A <5> (<5> vitamin K epoxidase activities are reduced in parallel with the carboxylase activities. Showed defects in the propeptide binding site. Slightly faster mobility than wild-type FLAG-CBX. CBX234/235 [2]) [2]
R359A/H360A/K361A <5> (<5> vitamin K epoxidase activities are reduced in parallel with the carboxylase activities. Showed defects in the propeptide binding site. CBX359/360/361 [2]) [2]
R406A/H408A <5> (<5> vitamin K epoxidase activities are reduced in parallel with the carboxylase activities. Showed defects in the propeptide binding site. CBX406/408 [2]) [2]
R416A/D417A <5> (<5> CBX416/417 [2]) [2]
R513A/K515A <5> (<5> vitamin K epoxidase activities are reduced in parallel with the carboxylase activities. They show normal affinity for the propeptide, FLEEL, proPT28, and vitamin K hydroquinone but exhibited a low catalytic rate for carboxylation. CBX513/515 [2]) [2]
R58G <2> (<2> warfarin resistent mutant [23]) [23]
R661A/R662A <5> (<5> CBX622/623/624 [2]) [2]
R671A/R672A/R673A <5> (<5> CBX671/672/673 [2]) [2]
R687A/K688A <5> (<5> CBX687/688 [2]) [2]
S398A <4> (<4> carboxylases W390A, S398A, and H404A have activities similar to that of wild type [20]) [20]
V29L <2> (<2> warfarin resistent mutant [23]) [23]
V45A <2> (<2> warfarin resistent mutant [23]) [23]
W390A <4> (<4> carboxylases W390A, S398A, and H404A have activities similar to that of wild type [20]) [20]
W399A <4> (<4> lower activity than wild type [20]) [20]
Y395A <4> (<4> lower activity than wild type [20]) [20]
Additional information <1,2,4,5> (<4> 38-BamHI site introduces 2 amino acid residues (glycine and serine) between the hGC fragment and the Lep tag. A 10-amino acid peptide (MDYKDDDDKG), including the FLAG epitope, is introduced to the amino-terminus of the full length of hGC to make FLAG-hGC and a 8-amino acid peptide (DYKDDDDK) is attached to the carboxyl-terminus of the full length of hGC to make hGC-FLAG. The FLAG-tagged hGC cDNA is subcloned into the EcoRI (Escherichia coli RY13) site of the expression vector pCl-neo under control of the cytωlovirus (CMV) promoter [12]; <1> analysis of a Ggcx+/- intercross reveals a partial developmental block with only 50% of expected Ggcx-/- offspring surviving to term, with the latter animals dying uniformly at birth of massive intra-abdominal he-

morrhage. This phenotype closely resembles the partial midembryonic loss and postnatal hemorrhage previously reported for both prothrombin and factor V (F5)-deficient mice. Ggcx-/-, dying uniformly at birth of massive intra-abdominal hemorrhage. Heterozygous mice carrying a null mutation at the γ-carboxylase (Ggcx) gene exhibit normal development and survival with no evidence of hemorrhage and normal functional activity of the vitamin K-dependent clotting factors IX, X, and prothrombin [11]; <2> N-terminal carboxylase peptide (residues 1-345) and the C-terminal peptide (345-758) two-chain form (residues 1-345 and residues 346-758) of the vitamin K-dependent γ-glutamyl carboxylase expressed in Sf9 insect cells. The carboxylase and epoxidase activities similar to those of one-chain carboxylase. The two-chain carboxylase is joined by a disulfide bond [9]; <5> R234A/H235A mutant, R406A/H408A mutant, and R513A/K515A mutant are more susceptible to inhibition by vitamin KH_2 than wild type enzyme. R234A/H235A mutant and R406A/H408A mutant exhibit maximal activity at 111 mM vitamin KH_2 and R513A/K515A mutant at 56 mM vitamin KH_2 [2]; <4> six out of seven patients with Pseudoxanthoma Elasticum habor mutations in the GGCX gene (γ-glutamyl carboxylase) [21]; <4> Y395A propeptide affinity is similar to that of wild type, but those of L394R and W399A are 16-22fold less than that of wild type. Results of kinetic studies with a propeptide-containing substrate are consistent with results of propeptide binding and FLEEL kinetics. Although propeptide and vitamin K binding in some mutants are affected, our data provide compelling evidence that glutamate recognition is the primary function of the conserved region around Leu394 [20]) [2,9,11,12,20,21]

Application

medicine <2,4> (<4> six out of seven patients with Pseudoxanthoma Elasticum habor mutations in the GGCX gene (γ-glutamyl carboxylase) [21]; <2> warfarin therapy [23]; <2> multiplexed single nucleotide polymorphism panel (interrogation of the CYP2C9 *2, *3, VKORC1 (-1639G3A), and GGCX (1181T3G) alleles simultaneously) can be successfully used in genotyping of patient blood samples, whereby results can be combined with other clinical parameters in an algorithm for warfarin dosing [30]) [21,23,30]

6 Stability

Temperature stability

37 <3> (<3> enzyme is not very stable at 37°C and lower temperatures are more desirable. At temperatures below 20°C, extended linear rates of incorporation of $^{14}CO_2$ into exogenous peptide substrates can be observed [4]) [4]

General stability information

<5>, freeze-thawing, three times, stable [16]

Storage stability

<5>, -70°C, 6 months, stable [16]

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