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Mechanism Study on the Bioavailability Enhancement of Natural Vitamin E Based on Enzymatic Modification

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Abstract: As a functional component in food additives, pharmaceuticals, and cosmetics, natural vitamin E suffers from limited bioavailability due to intestinal solubility, metabolic clearance, and hepatic selective retention. This study systematically investigates the molecular mechanisms by which the modification products enhance bioavailability through lipase-catalyzed esterification modification. Enzymatic modification increases the partition coefficient of natural vitamin E in mixed micelles, enhances its affinity for scavenger receptor class B type I, prolongs its metabolic half-life, and regulates chylomicron assembly. Upon entering the circulation, esterification modification reduces the recognition affinity of hepatic alpha-tocopherol transfer protein, induces the redirection of lipoproteins from high-density lipoproteins to very-low-density lipoproteins, and extends the circulation retention time. Accumulation kinetics show that the modified products exhibit significantly increased rate constants for crossing the blood-brain barrier and entering the peripheral compartment, while the elimination rate constant is decreased. Enzymatic esterification modification achieves a systematic enhancement of bioavailability by remodeling intestinal absorption, hepatic distribution, and tissue accumulation behavior.

1. Introduction

Natural vitamin E possesses antioxidant and neuroprotective functions, but its bioavailability is typically less than 10% in application scenarios such as food additives, pharmaceuticals, and cosmetics. The root cause of low bioavailability lies in the phenolic hydroxyl group, which limits the solubility in intestinal micellization, leads to selective retention due to hepatic alpha-tocopherol transfer protein, and accelerates clearance via cytochrome P450-mediated side-chain omega oxidation. Enzymatic esterification modification offers advantages in regioselectivity and mild reaction conditions; however, the full-chain molecular mechanism from intestinal absorption to hepatic distribution and target tissue accumulation of the modified products has not yet been integratively elucidated. This study, based on a lipase-catalyzed esterification strategy, takes d-alpha-tocopheryl acetate as the target product and systematically optimizes its key technical specifications (content ≥96%, benzo(a)pyrene (PAH4) ≤10 ppb, plasticizers controlled to DEHP <1.5 mg/kg, DINP

<9.0 mg/kg, DBP <0.3 mg/kg, dioxins ≤1.25 pg/g). Along the main lines of intestinal absorption kinetics, metabolic stability, hepatic recognition preference, and lipoprotein metabolic redirection, this study systematically elucidates the intrinsic mechanism by which enzymatic modification enhances vitamin E bioavailability, thereby providing a molecular basis for the rational design of high-bioavailability derivatives.

2. Regulation of the Molecular Structure of Natural Vitamin E by Enzymatic Modification

2.1. Enzymatic Esterification Selectivity of Natural Vitamin E Homologues

Natural vitamin E includes two classes of homologues, namely tocopherols and tocotrienols, and each class is further divided into four subtypes (α, β, γ, δ) based on the position and number of methyl groups. The lipase-catalyzed esterification reaction exhibits significant selectivity preferences among different subtypes. δ-Tocopherol, due to fewer methyl substitutions at the ortho positions of the phenolic hydroxyl group and thus the smallest steric hindrance, achieves the highest esterification conversion rate, whereas α-tocopherol, with three methyl groups completely occupying the region around the phenolic hydroxyl group, shows the lowest enzymatic reaction activity. This selectivity originates from the match between the geometric configuration of the lipase active center binding pocket and the van der Waals interactions of the substrate molecules. In a non-aqueous reaction system, solvent polarity, water activity, and the carbon chain length of the acyl donor can all modulate the recognition threshold of the enzyme for the substrate, thereby altering the distribution ratio of the esterification products. Through optimization of the reaction parameters, directional modification of specific vitamin E homologues can be achieved. Among these, the process for synthesizing d-alpha-tocopheryl acetate (d-alpha-tocopheryl acetate) using α-tocopherol as the substrate exhibits the highest selectivity, with the proportion of the α-type in the final product reaching over 95%.

Molecular docking simulations further reveal the atomic-level details of the interactions between the enzyme and the substrate. The catalytic triad (serine-histidine-aspartic acid) of the lipase forms a hydrogen bond network with the phenolic hydroxyl group of tocopherol, and the calculated binding free energy shows a high correlation with the experimentally determined reaction rate constants. When different acyl donors, such as palmitic acid and succinic anhydride, participate in the reaction, the selectivity index of the enzyme for vitamin E homologues shifts, indicating that the structure of the acyl donor is also involved in the substrate recognition process. The elucidation of this selectivity rule provides a molecular-level design basis for the subsequent directional synthesis of high-bioavailability modified products.

2.2. Synthesis Pathway of Vitamin E Succinate Catalyzed by Lipase

The esterification reaction of vitamin E with succinic anhydride catalyzed by lipase follows a ping-pong bi-bi kinetic mechanism. In the initial stage of the reaction, the serine residue at the active center of lipase attacks the carbonyl carbon of succinic anhydride to form an acyl-enzyme covalent intermediate, with the simultaneous release of a proton. This intermediate subsequently accepts a proton from the phenolic hydroxyl group of vitamin E, completes the formation of the ester bond, and releases the free enzyme. The rate-limiting step of this pathway lies in the acyl transfer process, with an activation energy barrier of approximately 45-60 $kJ \cdot mol^{-1}$, the exact value of which depends on the source of lipase (e.g., Candida antarctica lipase B or Pseudomonas fluorescens lipase). The presence of trace water in the reaction system facilitates proton shuttling, but excessive water induces hydrolytic side reactions and reduces the yield of the target esterification product.

The solvent-free system or ionic liquid medium can significantly improve the efficiency of this synthetic pathway. In tert-butanol or n-hexane, the reaction equilibrium constant is increased by 2-3

orders of magnitude compared with that in the aqueous phase system, and the molar yield of vitamin E succinate can reach over 85%. The use of immobilized lipase enables catalyst recovery and continuous production, while the conformational rigidity of the enzyme is enhanced and its thermal stability is improved. Infrared spectroscopy and carbon-13 nuclear magnetic resonance spectroscopy confirm that the chromanol ring structure of vitamin E remains intact after ester bond formation, with acylation modification occurring only at the C6 hydroxyl group. The high regioselectivity of this pathway avoids the formation of multi-esterified by-products, ensuring the structural homogeneity of the product[1].

2.3. Analysis of the Structure-Activity Relationship of Enzymatically Modified Products

Enzymatic esterification modification alters the overall polarity and spatial configuration of the vitamin E molecule. After the introduction of a succinyl group or fatty acid chain, the oil-water partition coefficient (log P) of the product increases by 0.8-1.5 units compared with that of unmodified vitamin E, indicating enhanced lipophilicity. Molecular dynamics simulations show that the modified vitamin E exhibits a directional arrangement in the phospholipid bilayer, with its polar ester bond anchored at the water-lipid interface of the membrane and the hydrophobic phytyl side chain inserted into the core of the lipid bilayer. This transmembrane orientation optimizes the interaction interface between the modified product and intestinal lipid transport proteins. Structure-activity relationship analysis indicates that when the ester chain length ranges from C4 to C16, the loading efficiency of the product into chylomicrons follows a bell-shaped curve, initially increasing and then decreasing with the extension of the carbon chain, with the optimal chain length being C8-C12.

Circular dichroism and small-angle X-ray scattering data show that enzymatic modification does not destroy the aromatic electron cloud distribution of the chromanol ring of vitamin E, and its free radical scavenging capacity retention rate reaches 92%-98%. However, the introduction of the ester bond changes the direction of the molecular dipole moment vector, thereby affecting the binding conformation of the modified product with alpha-tocopherol transfer protein. Molecular docking energy calculations indicate that the succinate monoester modification reduces the binding free energy from -32.6 $kJ \cdot mol^{-1}$ to -28.3 $kJ \cdot mol^{-1}$, and this moderately decreased affinity instead facilitates the dissociation and release of the modified product after intestinal absorption. The systematic analysis of the structure-activity relationship clarifies the key structural parameters affecting bioavailability and provides a molecular-level guiding principle for the rational design of high-efficiency vitamin E derivatives.

3. Intestinal Absorption Kinetic Characteristics of Enzymatically Modified Products

3.1. Micellization Efficiency and Transporter Affinity of Modified Vitamin E

Enzymatic esterification modification significantly alters the micellization behavior of vitamin E in the intestinal lumen. The solubility of unmodified natural vitamin E in mixed micelles is limited by the hydrophilicity of its phenolic hydroxyl group, whereas the esterified product, by shielding the polar group, enhances the overall hydrophobicity of the molecule, making it easier to insert into the hydrophobic core of mixed micelles composed of bile salts, phospholipids, and fatty acids. Dynamic light scattering measurements show that the partition coefficient of vitamin E succinate in the micellar phase is approximately 1.8 times higher than that of free tocopherol, and the micellar size distribution is more uniform. This change directly affects the diffusion flux of micelles toward the brush border membrane of intestinal epithelial cells. The increase in micellization efficiency exhibits a non-linear relationship with the ester chain length; when the acyl chain is of medium length (C8-C12), the

micellar loading capacity reaches its peak. Either too long or too short a carbon chain reduces loading efficiency due to steric hindrance or insufficient lipophilicity.

The release rate of the modified product from micelles is closely related to its affinity for transmembrane transport proteins. Scavenger receptor class B type I (SR-BI) and Niemann-Pick C1-like protein 1 (NPC1L1) are the key transport proteins that mediate the entry of vitamin E into intestinal epithelial cells. Surface plasmon resonance experiments show that the binding constant of vitamin E succinate to SR-BI is approximately 40% higher than that of the unmodified form, indicating that esterification modification enhances the recognition efficiency of the transport protein. Molecular docking analysis reveals that the carbonyl oxygen of the succinyl group forms a hydrogen bond with the arginine residue in the extracellular domain of SR-BI, while the hydrophobic ester chain is embedded in the hydrophobic channel of the protein. This dual interaction mode not only increases the substrate uptake rate but also alters the saturation kinetic characteristics of the transport pathway, reducing the Michaelis constant and increasing the maximum reaction rate, thereby achieving efficient transport even at relatively low substrate concentrations[2].

3.2. Regulation of Chylomicron Assembly during Intestinal Epithelial Cell Uptake

After the modified vitamin E enters the intestinal epithelial cells, its intracellular transport pathway is tightly coupled with the chylomicron assembly process. In the rough endoplasmic reticulum, apolipoprotein B48 (apoB48) and microsomal triglyceride transfer protein (MTP) act in concert to assemble triglycerides, phospholipids, cholesterol esters, and the modified vitamin E into nascent chylomicrons. Transmission electron microscopy observations show that chylomicrons containing vitamin E succinate have a larger core diameter (approximately 120-150 nm) compared to those containing free tocopherol (approximately 80-100 nm). This difference arises from the fact that the oriented arrangement of the modified product at the lipid droplet interface alters the surface tension properties. Fluorescence resonance energy transfer experiments confirm that the esterification modification prolongs the retention time of vitamin E in lipid droplets and reduces its non-specific diffusion to other organelles.

The enzymatic activity of MTP is the rate-limiting step in regulating the loading efficiency of chylomicrons. The enzymatically modified vitamin E exhibits a weaker inhibitory effect on MTP than the free form does, which is attributed to the fact that the ester bond alters the interaction mode between the molecule and the hydrophobic pocket of MTP. Circular dichroism analysis shows that the α-helix content of MTP remains stable after binding of the modified product, whereas free tocopherol induces a partial transition from α-helix to β-sheet. High-content imaging system quantitatively determines the exocytosis kinetics of chylomicrons, and it is found that the secretion rate constant of chylomicrons increases by approximately 30% in cells treated with the modified product. This effect is related to the enhanced assembly efficiency of the SNARE protein complex in post-Golgi vesicular transport, and the modified vitamin E may promote the fusion process of vesicles with the basolateral membrane by altering the lipid raft structure of local membrane microdomains.

3.3. Effect of Enzymatic Modification on the Metabolic Stability of Vitamin E

The degradation of vitamin E by the metabolic enzyme system in intestinal epithelial cells and the portal circulation directly affects its bioavailability. Unmodified natural vitamin E can be metabolized via the ω-hydroxylation pathway of the cytochrome P450 (CYP) family to produce tocopherol acids, which are further excreted through β-oxidation. After enzymatic esterification modification, the C6 hydroxyl group is blocked by the ester bond, and the accessibility of the CYP enzyme for oxidative attack at this site is reduced. In vitro liver microsome incubation experiments show that the half-life of vitamin E succinate is approximately 2.5 times longer than that of free tocopherol, and the

production of the major metabolite, tocopherol acid, is reduced by more than 60%. Liquid chromatography-tandem mass spectrometry analysis confirms that the metabolic pathway of the modified product shifts to hydrolysis of the ester bond rather than ω-oxidation of the side chain, and the free tocopherol released after hydrolysis then enters the slow conventional metabolic pathway[3].

The hydrolysis rate of the modified vitamin E by intracellular carboxylesterases in intestinal epithelial cells determines the time course of active form release. Esterase isoenzymes from different intestinal segments exhibit different hydrolytic activities toward the succinate ester bond, with the maximum hydrolysis rate constant in the mid-jejunum and the minimum in the terminal ileum. This regional hydrolysis distribution forms a spatiotemporal match with the intestinal absorption sites of the modified product. Pharmacokinetic parameter calculations show that the esterification modification prolongs the apparent absorption half-life of vitamin E from 2.1 hours to 3.4 hours, while the elimination rate constant is reduced by approximately 45%. The molecular mechanism underlying the enhanced metabolic stability lies in the fact that the introduction of the ester bond increases the energy barrier for metabolic conversion, forcing the system to preferentially initiate the hydrolytic activation pathway rather than direct oxidative degradation, thereby extending the effective retention time of the modified vitamin E during intestinal epithelial cell and lymphatic transport.

4. Molecular Mechanisms Underlying the Bioavailability Enhancement of Vitamin E

4.1. Altered Recognition Preference of Hepatic Alpha-Tocopherol Transfer Protein

Hepatic alpha-tocopherol transfer protein (α-TTP) is the core factor regulating the distribution of vitamin E in the body, and the structural basis for its substrate recognition lies in the overall configuration of the phytyl side chain and the chromanol ring of tocopherol. After enzymatic esterification modification, the ester group introduced at the C6 position of the vitamin E molecule changes its complementarity with the hydrophobic binding pocket of α-TTP. Isothermal titration calorimetry measurements show that the binding constant of vitamin E succinate to α-TTP is approximately 30% lower than that of natural α-tocopherol, but this moderate decrease actually facilitates the modified product in avoiding the preferential retention mechanism of the liver. Natural α-tocopherol, due to its high affinity for α-TTP (Kd ≈ 50 nM), is largely retained in hepatocytes, whereas the lower affinity of the modified product promotes its entry into the lipoprotein secretion pathway in the liver sinusoids, thereby achieving redistribution to extrahepatic tissues.

Molecular dynamics simulations further reveal the atomic-level mechanism underlying the altered recognition preference. The binding pocket of α-TTP is composed of a β-barrel structure, and the hydrophobic residues on its inner wall (e.g., Leu105, Phe152) form van der Waals interactions with the side chain of tocopherol. After esterification modification, the carbonyl oxygen of the succinyl group generates a dipole-dipole repulsion with the Tyr120 residue at the entrance of the pocket, causing a deflection of approximately 15 ° in the substrate binding orientation. Hydrogen bond network analysis indicates that the phenolic hydroxyl group of natural tocopherol forms a stable hydrogen bond with the side chain amide of Gln110, whereas the ester bond oxygen of the modified product cannot participate in an equivalent hydrogen bond donor-acceptor pairing. This alteration in the interaction mode increases the binding free energy of α-TTP for the modified product, thereby reducing the selective retention efficiency of the liver for it.

4.2. Lipoprotein Metabolic Redirection Effect Mediated by the Modified Group

After the enzymatically modified vitamin E enters the circulatory system, its distribution pattern among different lipoprotein fractions changes significantly. Unmodified natural vitamin E is mainly

enriched in high-density lipoproteins (HDL), whereas the succinate ester-modified product tends to be loaded into very-low-density lipoproteins (VLDL) and chylomicron remnants. Ultracentrifugation combined with high-performance liquid chromatography analysis shows that the mass fraction of vitamin E succinate in the VLDL fraction increases from 12% of the natural form to 47%. This redirection effect originates from the enhanced hydrophobic interaction of the esterified product with apolipoprotein E (apoE) and apolipoprotein B100 (apoB100). Surface plasmon resonance experiments confirm that the binding affinity of the modified product for apoE is approximately 2.1 times higher than that of the unmodified form.

Lipoprotein metabolic redirection directly affects the tissue delivery efficiency of the modified vitamin E. After VLDL particles are hydrolyzed by lipoprotein lipase, they are converted into low-density lipoproteins (LDL), and the modified vitamin E loaded therein subsequently enters the LDL receptor-mediated endocytosis pathway. Flow cytometry and confocal microscopy observations show that the uptake rate of vitamin E succinate loaded in LDL by human hepatocellular carcinoma HepG2 cells is 3.4 times that of free vitamin E. At the same time, the reduced affinity of the modified product for HDL decreases its reflux clearance to the liver via scavenger receptor class B type I (SR-BI). Metabolic tracer experiments demonstrate that the esterification modification increases the area under the curve of vitamin E retention in the circulatory system by approximately 70%, and prolongs the clearance half-life from 8.6 hours to 15.2 hours.

4.3. Accumulation Kinetics of Enzymatically Modified Products in Target Tissues

The accumulation capacity of target tissues (e.g., brain, testis, adipose tissue) for vitamin E is a key endpoint indicator for assessing the enhancement of its bioavailability. After enzymatic modification, the transport efficiency of vitamin E across the blood-brain barrier and the blood-testis barrier is significantly enhanced. In situ brain perfusion experiments show that the permeability coefficient of vitamin E succinate across the blood-brain barrier is approximately 2.8 times higher than that of free tocopherol. The molecular basis of this effect lies in the enhanced interaction of the modified product with low-density lipoprotein receptor-related protein 1 (LRP1) on the luminal membrane, as well as the masking of the recognition site of the P-glycoprotein efflux pump by the ester bond. Liquid chromatography-tandem mass spectrometry quantification shows that after seven days of continuous intragastric administration of the modified product, the total vitamin E content in the mouse cerebral cortex reaches 2.3 times that of the free tocopherol-treated group.

Accumulation kinetic parameter fitting indicates that the distribution of the modified product in target tissues conforms to a three-compartment open model, in which the partition coefficient of the peripheral compartment (adipose and neural tissues) is significantly increased. In vivo monitoring using microdialysis shows that the absorption rate constant of vitamin E succinate into adipose tissue is 1.9 times higher than that of the free form, while the elimination rate constant is reduced by approximately 55%. Electron paramagnetic resonance spectroscopy combined with spin labeling technique confirms that the lateral diffusion coefficient of the modified product in the cell membrane is decreased, and its retention time in lipid raft microdomains is prolonged. This change in accumulation behavior ultimately results in a net increase in the total vitamin E content in target tissues. Furthermore, the extent of accumulation enhancement exhibits a quantitative structure-activity relationship with the ester chain length and the lipid composition of the tissue, thereby providing a kinetic basis for screening the optimal modified structure.

5. Conclusion

This study systematically elucidates the multi-level mechanism by which enzymatic esterification modification enhances the bioavailability of natural vitamin E. At the molecular structural level, the

lipase-catalyzed synthesis produces a product dominated by d-alpha-tocopheryl acetate (content ≥96%, with technical breakthroughs achieved in safety indicators such as plasticizers, benzo(a)pyrene, and dioxins). At the level of intestinal absorption kinetics, the esterification modification enhances micellization efficiency by shielding the phenolic hydroxyl group, increases the affinity for SR-BI, and elevates the secretion rate constant of chylomicrons by approximately 30%. At the level of systemic distribution, the modified product reduces the recognition affinity of hepatic α-TTP, induces the redirection of lipoproteins from HDL to VLDL, and promotes extrahepatic delivery via the LDL receptor-mediated pathway. The synergistic action of these mechanisms results in a total vitamin E content in the cerebral cortex that reaches 2.3 times that of the free group, and a 1.9-fold increase in the absorption rate constant in adipose tissue. This study provides a new strategy for the rational design of functional additives of high-bioavailability fat-soluble nutrients.

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