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THE ROLE OF DEHYDROALANINES IN ENZYME AND PEPTIDE CHEMISTRY

ENZİM VE PEPTİD KİMYASINDA DEHİDROALANİNLERİN ROLÜ

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ABSTRACT

α,β-Dehydroamino acids represent an important class of compound as they are key-intermediates in amino acid and peptide synthesis and are constituents of a variety of naturally occurring antibiotic and phytotoxic peptides including several fungal metabolites which posses antibiotic activity. They also play an important role at the active site of some enzymes and show free radical scavenging activity. It is believed that more detailed investigations are necessary to understand the importance of dehydroalanines.

*Key **Words:** Dehydroalanine, Dehydration of serine, Lantibiotic, Antioxidant*

ÖZET

α,β-Dehidroamino asitler, peptid ve amino asit sentezinde anahtar ara ürün olarak, fitotoksik peptidler ve doğal antibiyotiklerin yapılarında bulunarak ve antibiyotik aktiviteye sahip olan fungal metabolitlerde yeralarak önemli bir biletik sınıfını oluşturmaktadırlar. Dehidroalaninler ayrıca bazı enzimlerin aktif bölgelerinde önemli rol oynar ve serbest radikal toplayıcısı olarak aktivite gösterirler. Daha detaylı araştırmalar yapılması dehidroalaninlerin önemlerinin vurgulanabilmesii için gerekli görülmektedir.

***Anahtar kelimeler:** Dehidroalanin, Serin dehidratasyonu, Lantibiyotik, Antioksidan*

INTRODUCTION

Dehydroamino acid occurred frequently in nature particularly in antibiotic peptides and plant pathogenic toxins. It has been postulated that the dehydroamino acids plays an important role giving the definite peptide conformation that is required for exhibition of biological activities (1) and could be an intermediate for the biosynthesis of an unusual amino acid or D-amino acid in many natural peptides.

Dehydroamino acids are important constituents of certain antibiotics(2) many of which have interesting physiological properties(3). The chemical reactivity of dehydroalanine generated from serine has been utilised for the site-specific cleavage of proteins(4). Dehydroalanine also plays a catalytic role in the active sites of some yeast and bacterial enzymes(5). Olefins such as dehydroalanines have been shown to inactivate free radicals by forming stabilised free radical adducts(6). Among these molecules N-acyl dehydroalanines react with scavenge oxygen and hydroxyl free radicals.

GENERAL SYNTHESIS OF DEHYDROALANINES

Considerable attention has been given recently to the preparation of dehydroamino acids, particularly dehydroalanine unit, which is generally derived from serine or cysteine derivatives. Several synthesis of dehydroamino acids have been reported. The most common ones are pointed in here.

Dehydroalanines can be synthesised using isourea-mediated β-elimination process from serine, cysteine and threonine(4).As a carbodiimide mostly dicycylohexylcarbodiimide (DCCI), diisopropylcarbodiimide (DiPCD) or water-soluble carbodiimide (WSC) is used usually in the presence of CuCl(7) (Figure 1). Peroxide-free solvents are required for the reaction and work-up since the unsaturated amino acid derivatives were found polymerise rapidly in the presence of trace amounts of peroxide.

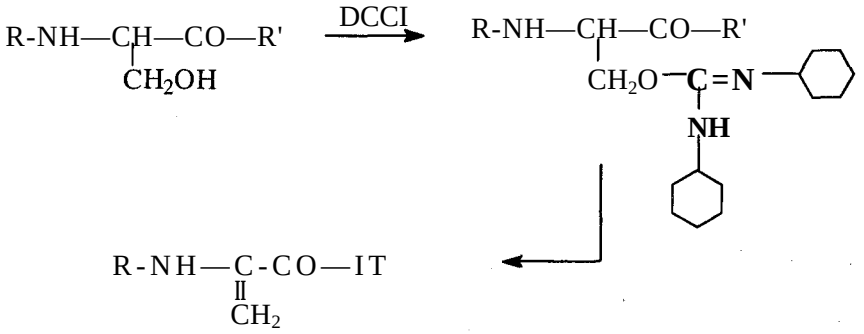


Figure 1. Dehydration of serine by DCCI

A number of reagent(8) including diethylchlorophosphate, oxalyl chloride, N,N'-carbonyldiimidazole and DAST (diethylaminosulfur trifluoride/pyridine) and several carbodiimides(9) have also been employed to perform the direct elimination.

One important and well-used approach involves the elimination reactions of serine derivatives containing suitable leaving groups(7,10). O-Tosylated (or O-mesylated etc.) serine derivatives undergo a β-elimination reaction on being treated with alkali in non-polar solvents, resulting the formation of dehydroalanine derivatives(l 1) (Figure 2). N-benzoylserine derivatives sometimes form oxazoline instead of the elimination product but N-benzyloxycarbonyl and N-acylserine derivatives give exclusively the elimination product(12).

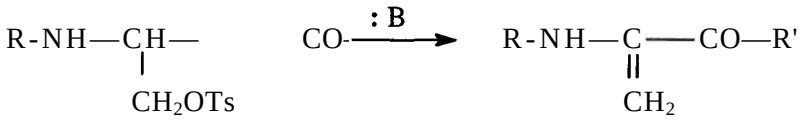
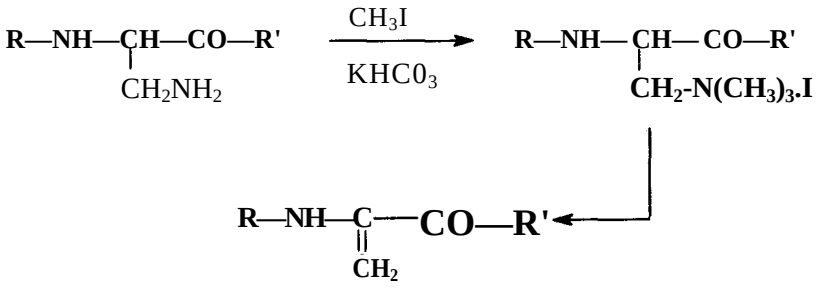


Figure 2. Tosyl elimination from serine derivative

An other novel method for the synthesis of dehydroalanine by means of Hoffman degradation of α,β-diaminopropionyl residue as shown below(14) (Figure 3).



Kolar et al(14) reported the preparation of dehydroalanines from N-acylamino esters by a sequence of N-chlorination and elimination (Figure 4).

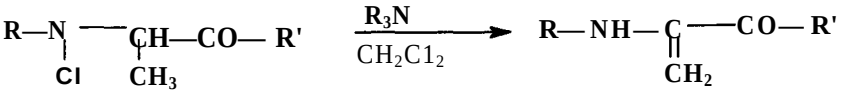


Figure 4. Preparation of dehydroalanine by Kolar et al(14)

DEHYDROALANINES IN PEPTIDE ANTIBIOTICS

Lantibiotics are antibiotic peptides that contain the rare thioether amino acids lanthionine and /or methyllanthionine. Some of the lantibiotics such as epidermin, Pep5 and epilancin K7 are produced by *Staphylococcus epidermis.* The biosynthesis of these lantibiotics proceeds from structural genes which code for prepeptides that are enzymatically modified to give the mature peptides. The genes involved in biosynthesis , processing, export etc. are found in gene clusters adjacent to the structural genes and code for transporters, immunity functions, regulatory proteins and modification enzymes LanB, LanC and LanD, which catalyse the biosynthesis of the rare amino acids. LanB and LanC are responsible for the dehydration of the serine and threonine residues to give dehydroalanine dehydrobutirine and subsequent addition of cysteine SH-groups to the dehydroamino acids which results in the thioether rings(15).

Dehydroalanines is present in a variety of peptide antibiotics of bacterial origin, including the "lantibiotics" nisin, subtilin, epidermin, gallidermin and more highly modified peptides such as thiostrepton, siomycin-A, nosiheptide and

berninamycin(5). Novel active antibiotics and immuno adjuvants have been synthesised by substituting dehydroalanine for other residues(16). Biosynthetically, the dehydroalanine in the lantibiotics originates from the dehydration of serine residues(17. The subsequent stereospecific intramolecular addition of thiols from cysteins generates the meso-lanthionine bridges which give the lantibiotics their name. The remaining dehydroalanines are important for biological activity although it is still unclear whether this is a conformational effect or is due to the chemical reactivity of the group(5).

Subtilin and nisin are gene-encoded antibiotic peptides that are ribosomally synthesised by *Bacillus subtilis* and *Lactococcus lactis,* respectively. Gene-encoded antibiotics are unique in that their structures can be manipulated by mutagenesis of their structural genes. Although subtilin and nisin share considerable structural homology, subtilin has a greater tendency than nisin to undergo spontaneous inactivation. This inactivation is accompanied by chemical modification of the dehydroalanine position 5(18).

Peptide antibiotic nisin produced by Lactococcus lactis, contains the uncommon amino acid residues, dehydroalanine and dehydrobutirine and five thio ether bridges(19). Chemical modification of nisin showed that the 2-hydroxyalanine-containing derivatives are spontaneously converted into the corresponding des-dehydroalanine derivatives which show a strong reduction in biological activity as compared to native nisin(20).

Thiostrepton that contains dehydroalanine moiety is a highly modified multicyclic peptide antibiotic synthesised by diverse bacteria. Although best known as an inhibitor of protein synthesis, thiostrepton is also a potent activator of gene expression in Streptomyces lividans. When the mechanism of action was investigated a covalent attachment of thiostrepton to tipA proteins mediated by bond formation between dehydroalanine of thiostrepton and cysteine of TipAS has been found(21).

Sublancin 168 is a novel lantibiotic produced by *Bacillus subtilis 168.* According to Paik et al(22) its behaviour during Edman sequence analysis and its NMR spectrum suggested that sublancin is a dehydroalanine-containing lantibiotic. The antimicrobial activity spectrum of sublancin is like other lantibiotics, inhibiting

Gram-positive bacteria but not Gram-negative bacteria; and like the lantibiotics nisin and subtilin in its ability to inhibit both bacterial spore outgrowth and vegetative growth. Subtilin is an extraordinarily stable lantibiotic showing no degradation or inactivation after being stored in aqueous solution at room temperature for two years. Sublancin will be an especially good model for studying the potential of lantibiotics as sources of novel biomaterials.

A10255 is a complex of new thiopeptide antibiotics characterised structurally by a cyclic peptide core to which is attached a side chain composed of dehydroalanine moieties(23). When the biosynthetic origin of antibiotic A10255 was investigated, the results demonstrate that it originates exclusively from amino acids in a. manner similar to the closely related thiopeptide antibiotics nosiheptide and thiostrepton(24)

Antibiotics A21459 A and B are homodetic cyclic peptides constituted by eight amino acid including dehydroalanine. This is a new inhibitor of bacterial protein synthesis and active against a few Gram-negative bacteria and Clostridium difficile.

Pachett et al(25) studied the conversion of (2-deutero-3-fluoro-D-Ala8)cyclosporin A to bioactive dehydroalanine analog (delta-Ala8)cyclosporin A. This dehydro compound is a useful intermediate for the preparation of position 8 analogs of cyclosporin A formed from it by the conjugate addition of thiol compounds.

When the structural preferences of dehydroalanine-containing peptides and model compounds investigated by X-ray crystallography, NMR, UV and IR spectroscopy, in all cases a nearly planar conformation was determined(26).

Asymmetric hydrogenation of the dehydroalanine residue was used to syntheses of gramicidin S analogs(27,28). It was found that these analogs containing α,β-dehydroalanine showed high antimicrobial activity especially against Gram-positive bacteria and appreciable activity against Gram-negative bacteria such as *E. coli* (29).

Lou et al[30] studied the structures and the isolation of berninamycins B, C, D from fermentation of *Streptomyces bernensis.* Berninamycin D has fewer

dehydroalanine units attached to the carboxyl carbon of the pyridine ring. Based on FAB-MS results, berninamycin C is postulated to have only one dehydroalanine unit attached to the carboxyl carbon of pyridine.

DEHYDROALANINES IN ENZYMES

Histidase is an enzyme that catalyses the deamination of L-histidine to trans-urocanic acid in the liver and skin of mammals. Dehydroalanine is present in the histidase from *Pseudomonas putida* and is distinct from the active site cysteine(31). Dehydroalanine also a catalytically important electrophilic centre for the enzyme(32). Histidase deficiency results in increased histidine and histamine in blood, and decreased uracanic acid in blood and skin. Formation of a dehydroalanine at the active site of histidase was found effective for human histidinemia(33).

The active sites of the enzyme phenylalanine ammonia-lyase (Pal) from Rhodosporidium toruloides contains a dehydroalanine residue that is believed to be essential for catalytic activity(34). A possible role of serine as a precursor of dehydroalanine at the active site of Pal also has been found(35). Furthermore, the dehydroalanine is believed to be added post-translationally as part of a prosthetic group covalently attached to the enzyme. Perhaps for this reason no attempts to produce Pal in foreign host cells have been reported.

Although most enzymes work in aqueous medium, at their active sites they can adjust the polarity to meet requirements of the reactions they catalyse. Thus a Friedel-Craft-type electrophilic substitution which is normally conducted in water-free media, can be used to activate the substrate for chemically difficult transformations. It is shown that histidine and phenylalanine ammonia lyase which contain the rare prosthetic group dehydroalanine, make use of a Friedel-Craft-type reaction which was thought to occur only in rather abiotic conditions. While histidine ammonia lyase catalyses the first step of histidine degradation in most cells, phenylalanine ammonia lyase is an important plant enzyme, producing cinnamic acid which is the precursor of lignins, cumarins and flavonoids(36).

The cAMP-dependent phosphorilation of related receptors for organic calcium channel blockers was studied by Rohrkaster et al(37). Tryptic peptide

analysis showed that cAMP-dependent protein kinase phosphorylates rapidly a serine in one peptide. Phospho serine was determined as the phenylthiohydantoin-derivative of dithiothreitol-dehydroalanine.

The tyroid couples two iodotyrosine molecules to produce thyroid hormon at the acceptor site in thyroglobulin, leaving dehydroalanine or pyruvate at the donor position(38). It was found that tyrosine 130 is an important donor of the outer iodothyronine ring and a donor for thyroxine formation in thyroglobulin.

DEHYDROALANINES AS FREE RADICAL SCAVENGERS

Free radicals are highly reactive species. They react with almost every type of cellular molecules (sugars, proteins, lipids and nucleotides), causing metabolic disturbances, cell injury and even cell death. Oxygen radicals are involved in tumor promotion and they are considered as the principle causative agents of the deleterious effects of ionising radiations. N-(acylaryl)-dehydroalanine derivatives called AD-compounds have shown inhibitory activity towards oxygen radicals(6). The o-methoxyphenylacetyl dehydroalanine derivative, indexed as AD-20 has free radical scavenging activity and protects against the effects of ionising radiation(39) (Figure 5).

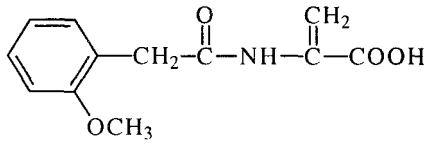


Figure 5. AD-20

CONCLUSION

Dehydroamino acids are constituents of an increasing number of antibiotic and phytotoxic natural products ranging in structural complexity from albonoursin to nisin, a 3500 molecular weight polypeptide containing dehydroalanyl residues. At least ten dehydroamino acid have been found in nature while these and other may serve as precursors for other unusual, naturally occurring amino acids. Protected α,β-

dehydroamino acids are valuable intermediates in the synthesis of bioactive dehydropeptides and uncommon or optically pure amino acids.

Although several methods for the synthesis of dehydroalanine peptides have been reported there are still problems to synthesise dehydroalanine derivatives in a peptide environment(7,40). The side chain of serine contains a primary hydroxy group while in threonine a secondary hydroxy group is present(41) and nucleophilic enough to involve with several different reactions such as acyl migration and intramolecular cyclisations.

Dehydroalanine derivatives occur widely in nature as constituent of peptides, proteins and enzymes. It has been postulated that the dehydroamino acids plays an important role in giving the definite peptide conformation that is required for exhibition of biological activities(13). Unsaturated sites in peptide hormones may act to enchange activity by virtue of an increased receptor binding affinity or by their ability to react irreversibly with a nucleophile on the receptor surface. It seems that the dehydroalanines will have more attention in the future since many antibiotics, enzymes and proteins are involved with this simple but important molecule.

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