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Transamination of the *N*-Terminal Residue of Carboxypeptidase

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The chemical evidence (Coombs, Omote & Vallee, 1964) that the essential zinc atom of carboxypeptidase A_γ is bound to the molecule through the *N*-terminal asparagine residue has been contradicted by the results of Ando & Fujioka (1962) and by X-ray-crystallographic data (Lipscomb *et al.* 1966; Reeke *et al.* 1967), which show the *N*-terminal residue of carboxypeptidase A_α to be far from the zinc atom. The results reported here confirm that the α-amino group of the *N*-terminal asparagine is not essential by showing that the enzyme remains fully active after transamination.

Dixon & Moret (1964) showed that the *N*-terminal residue of a protein can be transaminated into an α-oxoacyl residue. The best conditions for this (Dixon, 1967) are mild enough for the rest of the protein not to be greatly affected (van Heyningen & Dixon, 1967). The reaction appears specific for α-amino groups; the ϵ-amino groups of lysine residues are not transaminated, probably because a neighbouring peptide carbonyl is necessary to stabilize the reaction intermediate. The α-oxoacyl residue can be selectively cleaved from the rest of the molecule by treatment with bifunctional nucleophiles such as *o*-phenylenediamine (Dixon & Moret, 1964; van Heyningen & Dixon, 1967).

The transamination was carried out in a strong acetate buffer (in which carboxypeptidase is completely soluble) as high base concentrations are needed to catalyse the reaction (Dixon, 1967). The pH was about 5·4, which should be low enough for the binding of the zinc atom to be comparatively weak (Vallee, Rupley, Coombs & Neurath, 1960), so that an amino group bound to it might be available for reaction. Reaction was allowed to proceed for 26hr. as the dissociation of zinc is rather slow; results with other proteins show transamination itself to be complete in 20min. or so at room temperature. Carboxypeptidase (Calbiochem lot no. 72912) (0·55mg./ml.) was incubated at 1° for 26hr. in a solution containing 2M-sodium acetate, 0·4M-acetic acid, 0·1M-sodium glyoxylate and 5mM-$CuSO_4$. Controls, in which one or more of the reagents were omitted, were incubated under the same conditions. The solutions were then dialysed for 48hr. at 3° against M-NaCl, 0·1M-tris chloride, pH7·0, and 100μM-$ZnSO_4$ to restore activity to any apoenzyme formed. The solutions were then assayed for esterase activity by measuring the rate of release of H^+ ions from a solution of 5mM-hippuryl-DL-β-phenyl-lactate (in 25mM-LiCl and mM-phosphate buffer, pH7·5 at 25°; Simpson, Riordan & Vallee, 1963), and for peptidase activity by measuring the release of ninhydrin-positive material (assayed by the method of Rosen, 1957) from a solution of 10mM-benzyloxycarbonylglycyl-L-phenylalanine (in M-NaCl and 0·05M-tris chloride, pH7·5 at 25°; Coleman & Vallee, 1960). Protein concentrations were estimated from the extinction at 278mμ (Bargetzi, Sampath Kumar, Cox, Walsh & Neurath, 1963). Table 1 shows that the activities of the transaminated enzyme and controls are the same within about 3%, which is less than the experimental error. Peptidase activity fell off by about 25% on storage at 1° for 48hr, but the transaminated enzyme and controls had the same activity at any one time. Thus transamination has not affected the stability.

That the enzyme, incubated under conditions known to bring about transamination of some other proteins (Dixon 1967), had indeed transaminated was shown by two methods. (1) Treatment with dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride) (Gray & Hartley, 1963) followed by acid hydrolysis showed *N*-terminal aspartic acid (formed from asparagine) in the controls, but showed no *N*-terminus at all in the transaminated enzyme. All samples showed

Table 1. *Activity of carboxypeptidase A_γ after various incubations*

The complete medium was the only system in which transamination occurred. The error for inaccuracy of readings is thought to be <5% for the peptidase assay and <8% for the esterase assay.

Incubation medium	Peptidase activity (ΔE_{570}/min./mg.)	Esterase activity (μmoles/min./mg.)
Complete medium (see the text)	0·148	45·6
Complete medium less $CuSO_4$	0·146	46·5
Complete medium less sodium glyoxylate	0·152	45·5
Complete medium less $CuSO_4$ and sodium glyoxylate	0·150	44·6

O-dansyl-tyrosine and N^{ϵ}-dansyl-lysine, which are normally formed from residues within peptide chains. (Protein was incubated overnight at room temperature in a solution containing 50% acetone, 0·25M-$NaHCO_3$, 8M-urea and 10mg. of dansyl chloride/ml.) (2) When the transaminated enzyme was incubated under conditions that bring about the scission of terminal α-oxoacyl residues (van Heyningen & Dixon, 1967), the product had tyrosine as *N*-terminal residue. No tyrosine end group was produced when the native enzyme was incubated under the same conditions. Tyrosine is known to be the second residue in carboxypeptidase A_γ, a form of the enzyme that has *N*-terminal asparagine (Bargetzi *et al.* 1963). The activities of control and transaminated enzymes were completely destroyed by incubation under these conditions.

The fluorescence spectra (excitation at 289mμ) of the transaminated and native protein were determined. Addition of 8M-urea to a solution of the native enzyme shifted the maximum from 350 to 356mμ (uncorrected) and increased it by about 35%. On transamination, however, the maximum decreased by about 25% but did not shift. This shows that transamination has changed the environment of one or more of the tyrosine or tryptophan residues. It is obviously possible that the tyrosine residue adjacent to the *N*-terminus is at least partly responsible.

These experiments show that carboxypeptidase A_γ retains its activity when the *N*-terminal residue is transaminated into an α-oxoacyl residue.

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