Removal by Transamination and Scission of Residues from the Peptide Representing the Copper-Transport Site of Serum Albumin

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The peptide Asp-Ala-His-NH-Me was subjected to removal of its *N*-terminal residue by transamination and scission. Despite the high affinity of the peptide for Cu^{2+} ions, they catalysed its transamination smoothly. Two main transamination products were found, a complication previously observed with another peptide with an *N*-terminal aspartic residue, but their scission gave a single product, Ala-His-NH-Me. This was subjected to a further cycle of transamination and scission, and gave a single product after each step. For scission of transaminated peptides it proved unnecessary to remove them from transamination reagents provided that transamination was stopped with EDTA before adding the scission reagent.

Human serum albumin has one specific Cu^{2+} -binding site, and Cu^{2+} bound to this site is thought to be the transport form of Cu^{2+} in blood (Bearn & Kunkel, 1954; Neumann & Sass-Kortsak, 1967; Sarkar & Wigfield, 1968). The binding involves the *N*-terminal sequence Asp-Ala-His- and the atoms ligated to copper are the backbone nitrogen atoms of these three residues (including the terminal amino group) and the π -imidazole nitrogen of the histidine residue (Peters & Blumenstock, 1967; Shearer *et al.*, 1967; Bradshaw *et al.*, 1968; Bradshaw & Peters, 1969). Further, the peptide Asp-Ala-His-NH-Me binds Cu^{2+} ion in a square planar complex in the same way and with high affinity (Sarkar *et al.*, 1976; Sarkar, 1977; Iyer *et al.*, 1978). In order to study the effect of different sequences at the *N*-terminus of albumin it would be convenient to remove the natural sequence and replace it with other residues, if this could be done without denaturing the protein. Sequential removal of residues by transamination and scission (Dixon & Fields, 1972) seemed promising. The transamination step, however, depends on Cu^{2+} ions (although less satisfactory alternative catalysts can be used) and there was the fear that a peptide with a particularly high affinity for these ions might bind them in a manner that rendered them catalytically ineffective. In the present paper we report the stepwise removal of aspartic and alanine residues from the peptide Asp-Ala-His-NH-Me, and thus show that the strong affinity of this peptide for copper does not prevent its copper-catalysed transamination.

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Materials and Methods

Materials

Asp-Ala-His-NH-Me·HCl was synthesized by Iyer *et al.* (1978). *o*-Phenylenediamine was freshly crystallized from water. Sodium glyoxylate, purified by the method of Radin & Metzler (1955), was freshly dissolved.

High-voltage paper electrophoresis

This was performed at 110 V/cm in buffer solutions supported in Whatman 3MM paper cooled in white spirit. Three systems were used: pH 2 (8% acetic acid/2% formic acid); pH 3.5 (0.5% pyridine/5% acetic acid); pH 6.5 (10% pyridine/0.3% acetic acid; with 8% pyridine in the coolant). All percentages are by volume. Products were detected by ninhydrin stain, by Pauly's (1904) reagent (Bailey, 1967) and by staining with dinitrophenylhydrazine (5 mM in 2M-HCl).

Transamination

The peptide (5 mM) was incubated in a solution containing 2M-pyridine, 0.8M-acetic acid, 10mM-sodium glyoxylate and 2mM- CuSO_4 at room temperature (about 20°C) for 3 h (cf. Dixon & Fields, 1972). The reaction was stopped by addition of EDTA to 3 mM. Samples were taken for analysis by electrophoresis.

Scission

o-Phenylenediamine was added to 40 mM concentration, and acetic acid to make its total concentration 2M. Incubation then continued for 16 h and the products were separated.

Isolation of products

A total reaction volume of 1.25 ml was applied as a

line of 40 cm length to the Whatman 3 MM paper for electrophoresis at pH 3.5 for 15–20 min. At this loading separation proved good, and staining of guide strips allowed cutting out of the segment that contained the wanted product. This was eluted by 0.05 M-NH₃ solution and dried by rotary evaporation. The purity of the product was checked by electrophoresis at all three pH values, and by amino acid analysis.

Results

Transamination

Preliminary results showed that the standard transamination conditions (Dixon & Fields, 1972) of 0.1 M-sodium glyoxylate and 2 mM-CuSO₄ in a buffer of 2 M-pyridine and 0.8 M-acetic acid completely destroyed the original peptide in 3 h. The transamination, however, was unsatisfactory in that two major products, 1 and 2, were formed along with traces of minor ones. Both major products had the staining properties expected for the transaminated peptide (ninhydrin-negative, Pauly-positive and dinitrophenylhydrazine-positive). The relative mobilities of these products are given in Table 1(a).

It proved possible to stop the reaction by the addition of 3 mM-EDTA (there was no reaction when this was added initially), and such addition at 3 h was necessary to prevent the appearance of minor side products.

Since Dixon (1968) had reported complexities in the transamination of terminal aspartic residues, probably due to condensation of glyoxylate with the 3-carboxy-2-oxopropionyl residue formed (see the Discussion section), we tried the effect of changing the concentration of glyoxylate. Lowering it from 100 mM to 7 mM made the main product compound 1, and greatly decreased the amount of minor products, but left a little of the starting material unchanged

(not recovered by longer incubation). We finally settled for 10 mM-glyoxylate, which removed all starting material in 3 h. Variations of the buffer strength and substitution of formic acid for acetic acid led to no improvement; substitution of pyridoxal phosphate for glyoxylate and of NiSO₄ for CuSO₄ (cf. Dixon, 1967) greatly diminished the yield.

Since the conditions with 10 mM-glyoxylate gave conversion of all the starting material into products whose staining suggested they were 2-oxoacyl compounds, and would therefore be expected to undergo scission, we adopted them for routine use, especially as absence of a great excess of glyoxylate made the scission reaction easier.

Scission

Standard scission conditions (Dixon & Fields, 1972) were applied with the modification that the transamination products were not first isolated.

The scission conditions led to conversion of much of compounds 1 and 2 into material whose electrophoretic mobility (see Table 1a) was consistent with its gaining a positive charge, and which had retained Pauly-staining ability and gained ninhydrin-staining ability.

After electrophoretic isolation amino acid analysis gave no detectable aspartic acid, and a His:Ala ratio of 1.01:1. This was consistent with the desired product Ala-His-NH-Me.

Transamination and scission of Ala-His-NH-Me

A second cycle of transamination and scission reactions on 1 mM-peptide gave almost quantitative yields of the desired products, as judged from electrophoresis. The transamination product, presumably pyruvoyl-His-NH-Me, was ninhydrin-negative and Pauly-positive and possessed the mobilities given in Table 1(b). After scission it regained ninhydrin staining, and had mobilities (Table 1b) consistent

Table 1. *Electrophoretic mobilities of products*

Mobilities were measured from a zero of glucose to correct for electro-osmosis. They are given (a) relative to Asp-Ala-His-NH-Me and (b) relative to the product from the first round of transamination and scission, Ala-His-NH-Me.

Substance	pH	Mobilities ·			
		...	2	3.5	6.5
(a)					
Asp-Ala-His-NH-Me			(1)	(1)	(1)
Transamination product 1 (possibly CH ₃ -CO-CO-Ala-His-NH-Me)			0.6-0.7	1	1.0-1.1
Transamination product 2 (possibly HO ₂ C-CH ₂ -CO-CO-Ala-His-NH-Me)			0.4-0.5	0.3	-0.1
Scission product: Ala-His-NH-Me			1.2-1.3	1.6-1.8	2.3-2.4
(b)					
Ala-His-NH-Me			(1)	(1)	(1)
CH ₃ -CO-CO-His-NH-Me			0.5-0.6	0.5-0.6	0.5
His-NH-Me			1.1	1.2	1.1

with its being histidine methylamide. Amino acid analysis showed only histidine.

Discussion

The successful transamination of Asp-Ala-His-NH-Me shows that its ability to bind Cu^{2+} ion does not inhibit its Cu^{2+} -catalysed transamination. Two products were formed just as from *Pseudomonas aeruginosa* cytochrome *c*-551 from which the *N*-terminal glutamic residue had been removed and whose new *N*-terminal residue was aspartic acid (Dixon, 1968). The two products were ketonic and did not appear to interconvert, so the simplest explanation is that the $\text{HO}_2\text{C}-\text{CH}_2-\text{CO}-\text{CO}-$ group can undergo a metal-ion-catalysed decarboxylation (cf. Krebs, 1942). Although the mobilities of the two products are consistent with this, i.e. that product 1 is $\text{CH}_3-\text{CO}-\text{CO}-\text{Ala}-\text{His}-\text{NH}-\text{Me}$ and that product 2 is $\text{HO}_2\text{C}-\text{CH}_2-\text{CO}-\text{CO}-\text{Ala}-\text{His}-\text{NH}-\text{Me}$, no firm evidence has been obtained. An alternative possibility is that in the presence of metal ions the methylene group of $\text{HO}_2\text{C}-\text{CH}_2-\text{CO}-\text{CO}-$ is reactive and may condense with glyoxylate (cf. Ruffo *et al.*, 1962), and the resulting product may or may not decarboxylate as described above, but we consider this to be less likely in view of the observed mobilities.

These results also show that there is no need to remove a peptide from transamination reagents before the scission reaction, since EDTA proved sufficient to stop all further transamination, and no interference with scission was found.

Since the peptide that binds Cu^{2+} ions so tightly nevertheless underwent smooth Cu^{2+} -catalysed transamination, the way is open for applying this reaction sequence to proteins, such as serum albumin, that have an *N*-terminus with similar binding properties.

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