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1. Bezzi, S., Mammi, M. and Garbuglio, C. *Nature* **182** (1958) 247.
2. Bezzi, S., Garbuglio, C., Mammi, M. and Traverso, G. *Gazz. Chim. Ital.* **88** (1958) 1226.
3. Mammi, M., Bardi, R., Garbuglio, C. and Bezzi, S. *Acta Cryst.* **13** (1960) 1048.
4. Hordvik, A., Sletten, E. and Sletten, J. *Acta Chem. Scand.* **20** (1966) 2001, and *to be published.*
5. Johnson, S. M., Newton, M. G., Paul, I. C., Beer, R. J. S. and Cartwright, D. *Chem. Commun.* **1967** 1170.
6. van den Hende, J. and Klingsberg, E. *J. Am. Chem. Soc.* **88** (1966) 5045.
7. Giacometti, G. and Rigatti, G. *J. Chem. Phys.* **30** (1959) 1633.

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Conversion of α-L-Angiotensinamide to β-L-Angiotensin during Enzymatic Degradation

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Earlier studies by Sjöholm and Yman [1] on the inactivation of α-L-angiotensinamide* by oxytocinase showed a rapid degradation of the peptide. However, the pressor activity of the incubation mixture always followed a characteristic course, indicating that a biologically active intermediate could have been formed during incubation. The present communication describes the electrophoretic separation of the vasopressor components of the mixture obtained after incubation of α-L-angiotensinamide with oxytocinase prepared according to Sjöholm *et al.*[2,3]

Experimental and results. α-L-Angiotensinamide (2 mg) was dissolved in 300 μl of sodium phosphate buffer (0.005 M, pH 7.4) and digested with 100 μl of oxytocinase (dialyzed against 0.001 M phosphate buffer, pH 7.4) at 37°C for 24 h. A control sample, in which the enzyme was replaced by the same amount of water, was treated in the same way. After incubation, 50 μl of the test and control samples were used for high-voltage electrophoresis on Whatman 3 MM paper, together with references (see Fig. 1). The references were detected with ninhydrin reagent, after which the paper was cut into sections and eluted with 0.9 % sodium chloride for 15 h. The hypertensive activity of the fractions was determined in a male rat, with β-L-angiotensin as reference, in a 4-point assay. In the test sample, biological activity

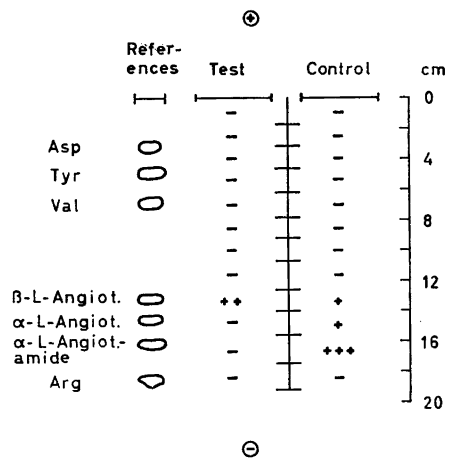

Fig. 1. High-voltage paper electrophoresis (1500 V, 2 h) in formic acid-pyridine buffer (1 ml of pyridine and about 20 ml of formic acid in 2000 ml of water to pH 2.7). The references were stained with ninhydrin reagent. The signs in the areas from the test and control samples denote the amount of biological activity present.

* We gratefully acknowledge the gift of α-L- and α-D-angiotensin (α-L- and α-D-Asp[1]-Val[5]-angiotensin-II), β-L-angiotensin (β-L-Asp[1]-Val[5]-angiotensin-II), and α-L-angiotensinamide (α-L-Asp(NH_2)[1]-Val[5]-angiotensin-II) from Dr. B. Riniker, CIBA Ltd, Basle.

was found only in the place of β-L-angiotensin, and in the control in the place of α-L-angiotensinamide, α-D,L-angiotensin, and β-L-angiotensin. Since small amounts of α-angiotensinamide and α-angiotensin could have contaminated the β-angiotensin extracts by trailing during electrophoresis, these were further treated with oxytocinase for 20 h, to destroy the last traces of α-angiotensinamide and α-angiotensin, and submitted to a renewed biological determination in the rat. The activity corresponded to 73 and 36 μg of β-angiotensin in the test and control sample, respectively. The β-angiotensin content in the control corresponded reasonably well to the amount of impurities present in the α-L-angiotensinamide used, as stated by the producer. Thus, at least 2 % of α-L-angiotensin—if the yield in the electrophoresis step is supposed to be 100 %—is transformed into β-angiotensin during degradation with oxytocinase.

Discussion. It has earlier been shown that β-L-angiotensin—in which the N-terminally situated aspartyl residue is bound *via* its β-carboxyl group to the rest of the peptide chain—and α-D-angiotensin are not degraded by oxytocinase [1] and other aminopeptidases.[4–6] This fact provides a basis for a study of the possible transformation or racemization of the N-terminal aspartyl residue [2] to the β-form or D-form, respectively, during incubation with aminopeptidases like oxytocinase. Buchanan *et al.*[7] found that β-aspartyl peptides are excreted in the urine and it was later shown by Haley *et al.* that β-aspartylglycine—and, in limited amounts, also β-aspartylalanine—were formed during exhaustive enzymatic degradation of human haemoglobin,[8] bovine ribonuclease [9] and other proteins. However, the spontaneous transformation of α-asparaginyl bonds to the corresponding β-aspartyl necessitates parallel control incubations without enzymes.

The present study showed that transformation of the peptide linkage from the α-carboxyl group to the β-carboxyl also occurs when the penultimate residue is a basic amino acid, suggesting a more general validity of the transformation reaction than earlier found. Haley *et al.*[8,9] used a somewhat complex mixture of endopeptidases and exopeptidases in their degradations. The present preliminary work shows that the use of an aminopeptidase is sufficient to yield an α-β transformation. However, whether the enzyme is the primary cause of the transformation, or is secondary to the hydrolytic cleavage, cannot at present be settled. Haley and Corcoran [9] found that the β-asp-gly formation was independent of the enzyme concentration, but increased with the incubation time.

The electrophoretic technique used does not allow the separation of β-L- and β-D-angiotensin and therefore—as the racemic form also has high hypertensive activity—the α-β transformation might be accompanied by a racemization. However, when the degradation of α-L-angiotensinamide with oxytocinase was performed in tritiated water with high specific radioactivity, and the mixture analyzed by electrophoresis, no radioactivity was incorporated in the β-angiotensin, which should be the case if an inversion reaction involving the asymmetric carbon atom had taken place.

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1. Sjöholm, I. and Yman, L. *Acta Pharm. Suecica* **4** (1967) 65.
2. Sjöholm, I., Yman, L. and Sandberg, F. *Acta Pharm. Suecica* **2** (1965) 261.
3. Sjöholm, I. and Yman, L. *Acta Pharm. Suecica* **3** (1966) 377.
4. Bumpus, F. M., Smeby, R. R., Page, I. H. and Khairallah, P. A. *Can. Med. Ass. J.* **90** (1964) 190.
5. Regoli, D., Riniker, B. and Brunner, U. *Biochem. Pharmacol.* **12** (1963) 63.
6. Riniker, B. *Metabolism* **13** (1964) 1247.
7. Buchanan, D. L., Haley, E. E., Markiw, R. T. and Peterson, A. A. *Biochemistry* **1** (1962) 620.
8. Haley, E. E., Corcoran, B. J., Dorer, F. E. and Buchanan, D. L. *Biochemistry* **5** (1966) 3229.
9. Haley, E. E. and Corcoran, B. J. *Biochemistry* **6** (1967) 2668.

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