Short Communications

The Enzymic and Non-enzymic Cleavage of *N*-Terminal L-Serine *O*-Sulphate

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It is now well established that a system specifically catalysing the $\alpha\beta$ -elimination of L-serine *O*-sulphate is widely distributed in Nature (Dodgson & Tudball, 1961). Moreover the system is unusual in that it does not require the participation of pyridoxal phosphate as an obligatory cofactor (Tudball, Thomas & Fowler, 1969). Even though the enzyme-catalysed reaction occurs without a requirement for pyridoxal phosphate, it has been demonstrated that a similar reaction is brought about when the substrate is incubated in the presence of pyridoxal phosphate and metal ions (Thomas, Dodgson & Tudball, 1968).

A great deal of information, particularly on the mechanism of enzyme action in the field of amino acid metabolism, has been accumulated as the result of investigations into model reactions corresponding to the appropriate enzyme-catalysed reactions under investigation. Since there has been no previous report on the interaction of pyridoxal and its derivatives with peptide-bound forms of substituted amino acids it became necessary to carry out investigations on the chemically catalysed degradation of such dipeptide derivatives as part of our continuing programme of study into systems that effect the degradation of L-serine *O*-sulphate and related compounds.

Incubation of L-serylglycine *O*-sulphate (final concentration 10 mM) at 50°C for 1 h with cupric chloride and pyridoxal (1 mM and 2 mM final concentrations respectively) in 0.02 M-sodium maleate-maleic acid buffer, pH 7.0, yielded SO_4^{2-} , NH_4^+ and an oxo acid as products. Isolation of the oxo acid was achieved by chromatographing the incubation mixture (10 ml) on a column (1.4 cm $\times$ 80 cm) of the strongly basic resin De-Acidite FF-IP, equilibrated and developed with a solution of sodium acetate (0.5 M), acetic acid (0.5 M) and toluene (0.03%) (Dixon, 1964). Detection and subsequent isolation of the oxo acid was essentially performed as described by Dixon (1964). Recrystallization of the resulting material from ethyl acetate-cyclohexane yielded a product (4.4 mg; 30% yield) having

m.p. 89°C, which corresponds to that reported for *N*-pyruvoylglycine by Bergmann & Grafe (1930).

Further identification of the product was made by electrophoresis on Whatman no. 1 paper in formic acid-acetic acid buffer, pH 1.9, before and after hydrolysis in 2 M-hydrochloric acid at 100°C for 16 h. Before hydrolysis the material migrated separately from pyruvate, serine and glycine, was ninhydrin-negative but did possess a reactive carbonyl function. After hydrolysis two compounds were obtained that migrated with mobilities identical with those of glycine and pyruvate. It was concluded that the original material was *N*-pyruvoylglycine, which received further substantiation when the isolated material was compared with an authentic sample. Mixed melting-point determinations, chromatographic, electrophoretic and spectrophotometric examination, coupled with elementary analysis, all indicated that the oxo acid isolated from the incubation mixture was *N*-pyruvoylglycine. This material was also detected as a product when L-serylglycine was present in the incubation medium in place of the sulphate ester, but the overall reaction occurred at a much lower rate. In cases where L-serine *O*-sulphate was the *N*-terminal residue in dipeptides formed with phenylalanine, leucine, valine and alanine it again proved possible to isolate an *N*-pyruvoyl-amino acid after incubation with pyridoxal and Cu^{2+} . However, no such reaction occurred when L-serine *O*-sulphate became a *C*-terminal residue, as in glycyl-L-serine *O*-sulphate.

The spectral changes associated with the reaction between L-serylglycine *O*-sulphate, pyridoxal and Cu^{2+} are illustrated in Fig. 1. Spectra were recorded by using a Unicam SP.800 spectrophotometer fitted with a thermostatically controlled cell housing. The extinction at 316 nm due to pyridoxal decreased with time and was closely paralleled by an increase in extinction at 384 nm. The two distinct peaks with maxima at 220 nm and 252 nm were replaced by a very broad peak in the region 200–250 nm. These spectral changes are similar to those

Fig. 1. Spectral changes occurring in a solution containing 5 mM-L-serylglycine *O*-sulphate, 0.2 mM-pyridoxal and 0.2 mM-CuCl₂ in 20 mM-Na₂HPO₄-NaH₂PO₄ buffer, pH 7.0, at 50°C. Curve 1 was recorded immediately after the reactants had been mixed. The remaining curves up to curve 2 were recorded 10, 20, 30 and 40 min later. Measurements were made with a Unicam SP.800 spectrophotometer (light-path 10 mm).

observed with L-serine *O*-sulphate (Thomas *et al.* 1968). The appearance of an absorption peak with a maximum at 384 nm is also similar to that described for a system consisting of pyridoxal, Al³⁺ and a number of dipeptides (Cennamo, 1967), and has been ascribed to the formation of a metal chelate of an imine derived from pyridoxal, metal ions and α -amino acids (probably in the proportions 1:1:1) (Davis, Roddy & Metzler, 1961). In the absence of metal ions this reaction occurs to a very limited extent. It has been suggested that these complexes are intermediates in such systems (Metzler, Ikawa & Snell, 1954; Snell, 1958). It is worth noting, however, that when glycyl-L-serine *O*-sulphate is incubated in the presence of pyridoxal and Cu²⁺ then similar spectral changes to those observed above occur, but no corresponding degradation occurs.

The activity of a highly purified preparation of pig liver enzyme (Tudball, Thomas & Bailey-Wood, 1971) that degrades L-serine *O*-sulphate was shown in most respects to parallel the activities observed with pyridoxal. Under the experimental conditions described by Thomas & Tudball (1967) only dipeptides containing *N*-terminal L-serine *O*-sulphate residues were attacked. When L-serine *O*-sulphate was the *N*-terminal residue linked to alanine, glycine, valine, leucine and phenylalanine, then 0.41, 0.37, 0.18, 0.08 and 0.02 μ mol of SO₄²⁻ respectively were liberated under the above conditions from 11 μ mol of substrate. No activity was detected towards glycyl-L-serine *O*-phosphate. It was concluded that, in addition to inorganic SO₄²⁻, peptides consisting of pyruvate and the appropriate

amino acid were produced. The pyruvate-linked amino acids were detected by paper electrophoresis as described above and by paper chromatography according to the method of Wieland, Shin & Heinke (1958). The products were located with either ninhydrin or 2,4-dinitrophenylhydrazine. The products, which all contained a reactive carbonyl function and a free amino group, migrated separately from pyruvate, serine and the other amino acid associated with the parent dipeptide sulphate. As a check on the validity of these observations, pyruvoylglycine was isolated after the incubation of enzyme and L-serylglycine *O*-sulphate. The enzyme preparation (2 ml containing 5 mg of protein/ml) was incubated for 3 h at 37°C with an equal volume of 0.1 M-dipeptide solution in 0.1 M-sodium acetate-acetic acid buffer, pH 7.0 (containing a final concentration of 5 mM-2-mercaptoethanol). Enzyme action was stopped by the addition of 4 M-perchloric acid (1 ml). The precipitated protein was removed by centrifuging and the supernatant adjusted to pH 7.0 with 30% (w/v) potassium hydroxide. The precipitated potassium perchlorate was removed by centrifuging and the supernatant employed for the isolation of pyruvoylglycine as described by Dixon (1964). The resulting product (3 mg; 10% yield) had m.p. 89°C and migrated coincidentally with authentic pyruvoylglycine on paper chromatography and paper electrophoresis under the conditions described above. Equivalent amounts of SO₄²⁻, NH₄⁺ and pyruvoylglycine measured by the method of Dixon (1964) were further shown to be liberated in the enzyme-catalysed reaction.

The observations presented in this communication provide a novel and easy method for the preparation of *N*-pyruvoyl-amino acids. It has further been observed, as would be predicted, that other electronegative substituents may replace the sulphate ester grouping on the serine residue.

The overall pattern of the degradation of *N*-terminal L-serine *O*-sulphate residues is the same when either pyridoxal or enzyme is employed as the catalyst. It is not possible at this stage, however, to ascertain whether the mechanisms responsible for these degradations are similar. A pyridoxal phosphate-independent histidine decarboxylase of *Lactobacillus* 30a (Riley & Snell, 1968) has been isolated in which the role of the normal cofactor is assumed by a pyruvic acid residue. It is doubtful whether a similar situation exists in the present case since a number of well-known carbonyl reagents had little or no effect on the rat liver enzyme (Thomas & Tudball, 1967).

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