The Conversion of Glyoxyloyl Groups into Glycyl Groups and their Formation from Maleyl Residues

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(Received 20 December 1967)

Dixon & Weitkamp (1962) converted the *N*-terminal serine residue of corticotropin into a glycine residue by oxidizing it with periodate to form a glyoxyloyl group, which was reductively aminated by transamination for 2 hr. at 50°. Milder conditions of transamination than were then known have since been used for converting α -aminoacyl groups into α -oxoacyl groups (e.g. Dixon, 1967), so it seemed worth while to test whether a glyoxyloyl group could be converted into a glycyl group in similarly mild conditions.

N α -Glyoxyloylarginine was used as a model compound, largely because of the ease of detecting arginine derivatives on paper by the Sakaguchi reaction. It was treated at room temperature with 0.2M-glutamic acid (0.1M excess) in *m*-pyridine in the presence of 0.01M-nickel sulphate for 30 min., and conversion into glycylarginine was virtually complete. This was isolated in an overall yield of 60%, calculated from the arginine used for preparing the model compound.

The function of each component is explained if transamination is due to conversion of structure (I) into structure (II) (where $R=[CH_2]_2 \cdot CO_2H$ and $R'=CH(CO_2H) \cdot [CH_2]_3 \cdot NH \cdot C(NH_2) : NH$ in the case described), by analogy with other transaminations. The role of pyridine is presumably removal of the α -proton of the glutamic acid. In other transaminations acetate may be used as the base, but here pyridine is more effective and is easily removed by evaporation.

Since maleic anhydride reacts smoothly with amino groups (Butler, Harris, Hartley & Leberman, 1967), it could provide a method of adding glyoxyloyl groups if the maleyl derivative could be oxidized. Pappo, Allen, Lemieux & Johnson (1956) reported that periodate in the presence of catalytic amounts of osmium tetroxide cleaved olefins to form aldehydes. Burton & Riley (1966) and Burton

(1967) showed that osmium tetroxide reacted only slowly unless NH_3 was present. D. M. Brown & B. E. Looker (unpublished work) found that at pH values high enough for appreciable amounts of free NH_3 to exist periodate failed to cleave osmate esters. They therefore used stoichiometric quantities of osmium tetroxide to obtain the osmate esters of olefins, and subsequently cleaved these esters with periodate at a lower pH. For the present purpose, however, quite small rates of oxidation were acceptable. At pH 3.5 the oxidation of maleylarginine to glyoxyloylarginine was found to be almost complete in 2 hr. at 37° when a slight excess of periodate over the stoichiometric requirement (including that for oxidizing the free glyoxylate formed) was added, together with 0.02 mole of osmium tetroxide/mole of maleyl compound. When ruthenium dioxide was added in place of osmium tetroxide (cf. Nakata, 1963), glyoxyloylarginine was again formed, at either pH 3.5 or pH 6. The reaction was less complete than with osmium tetroxide, but optimum conditions have not been sought in this case.

The overall reaction is as follows:

The conditions of oxidation of the maleyl group are fairly severe, so this reaction may be applicable only to fairly simple compounds. The conditions of transamination of the glyoxyloyl groups, however, are mild, and may therefore be used for converting *N*-terminal serine or threonine residues of proteins into glycyl groups with the minimum danger of side reactions, including denaturation.

Experimental. (a) Analysis of arginine derivatives. Samples (5 or 10 μ l.) were submitted to paper electrophoresis at pH 3.5 in pyridine-acetate buffer, with white spirit as coolant (cf. Ambler,

1963). The papers were examined by the Sakaguchi reaction (Jepson & Smith, 1953) and sometimes by reaction with ninhydrin (Heilmann, Barrolier & Watzke, 1957) or 2,4-dinitrophenylhydrazine (Bland, 1949).

(b) Preparation of maleylarginine. L-Arginine monohydrochloride (1.05g., 5m-moles) and maleic anhydride (0.60g., 6m-moles) were dissolved in 5ml. of water and rapidly adjusted to pH 6 with N-NaOH. The expected quantity of alkali, 12ml., was required, because at room temperature the maleic anhydride reacts rapidly with even the small fraction of α -amino group unprotonated below pH 6. Analysis by paper electrophoresis showed almost complete disappearance of arginine and appearance of a spot that was almost neutral at pH 3.5. This was Sakaguchi-positive and ninhydrin-negative.

(c) Oxidation of maleylarginine. The pH of the solution was adjusted to 3.5 with N-HCl (4.7ml. required) and sodium metaperiodate (4.28g., 20m-moles) was added. The calculated quantity was 19m-moles, 12m-moles to react with the double bond and 7m-moles for the further oxidation of the free glyoxylate formed (cf. Sprinson & Chargaff, 1946), of which 5m-moles would arise from one end of the maleyl group and 2m-moles from the free maleate. Osmium tetroxide solution (100 μ moles in about 1ml. of water), standardized spectrophotometrically as described by Burton & Riley (1966), was added and the mixture was stirred at 37°. Analysis by electrophoresis showed that little maleylarginine remained after 2hr., but that a new Sakaguchi-positive substance was formed. It possessed about a third of the cationic mobility of arginine at pH 3.5, was ninhydrin-negative and gave a yellow spot on treatment with dinitrophenylhydrazine. These properties and its subsequent transamination characterized it as glyoxyloylarginine.

(d) Isolation of glyoxyloylarginine. To remove most of the sodium iodate formed, part of which was precipitated during the reaction, the solution was cooled, 3 vol. of 96% (v/v) ethanol was added and the precipitate was filtered off and washed with a little 72% (v/v) ethanol. The filtrate was concentrated to a thick syrup by rotary evaporation and dissolved in 20ml. of a buffer of 0.1M-pyridine and 0.025M-acetic acid. It was then applied to a column (about 30cm. $\times$ 2cm.) of a sulphonated polystyrene resin (Zeo-Karb 225 SRC-16; < 200 mesh; 8% cross-linking) that had been equilibrated with a buffer of 0.2M-pyridine and 0.05M-acetic acid. It was eluted with this buffer at about 1ml./min. Maleylarginine emerged almost unretarded (0.5 column volume), glyoxyloylarginine emerged at 1-2 column volumes and arginine and Na⁺ did not emerge within 4 column volumes.

Glyoxyloylarginine was recovered from the fractions that contained it (as shown by paper electrophoresis) by rotary evaporation.

(e) Preparation of glycylarginine. The syrup of glyoxyloylarginine derived from 5m-moles of arginine was dissolved in 50ml. of a solution of pyridine (1M), L-glutamic acid (0.2M) and nickel sulphate (0.01M). After 30min. at room temperature, analysis by paper electrophoresis showed that almost all the Sakaguchi-positive material had been converted into a substance that moved almost as fast as arginine at pH 3.5. On reaction with ninhydrin in the presence of cadmium acetate it gave the yellow colour characteristic of peptides that have N-terminal glycine. In these properties it was the same (R. W. Carrell, personal communication) as glycylarginine isolated from an abnormal haemoglobin (Sansone, Carrell & Lehmann, 1967).

(f) Isolation of glycylarginine. Water and excess of pyridine were removed by rotary evaporation, and the solid residue was suspended in 15ml. of water and adjusted with N-NH₃ to pH about 8, when it dissolved. It was then applied to a column (25cm. $\times$ 2cm.) of sulphonated polystyrene beads (Zeo-Karb 225 SRC-16) that was equilibrated and developed with 0.3M-NH₄HCO₃. Glutamic acid and much of the Ni²⁺ emerged unretarded (0.5 column volume), some pyridine, evidently retained by glutamic acid on drying, emerged at 1.7-2.0 column volumes and the product emerged at 1.3-4.5 column volumes. The fractions positive by Sakaguchi or ninhydrin reactions of 10 μ l. spots on paper were pooled and evaporated to dryness. A little water was added to dissolve the remaining NH₄HCO₃, which was removed by subjecting the sample to a pressure of about 15 torr. overnight in a desiccator that contained vessels of dilute H₂SO₄ and dilute NaOH. Maintenance of the pressure of water vapour prevented the formation of crystalline NH₄HCO₃, which is non-volatile when dry. Final drying gave a glass of the peptide, partly as its bicarbonate. This was dissolved in water and titrated with N-HCl to pH 4.5, which gave a rough measure of the yield. Samples were hydrolysed with HCl, and arginine determination (Sakaguchi, 1950) showed a yield of 3.0m-moles (60%).

I thank Dr D. M. Brown for helpful advice and discussion, and him and Dr B. E. Looker for their unpublished results. I thank Mrs G. R. Law for her careful and skilled assistance, and Professor F. G. Young for his interest.

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