Chemical Modification of Arginine with 1,2-Cyclohexanedione

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A new α-amino acid was prepared by the reaction of 1,2-cyclohexanedione (I) with L-arginine in aqueous 0.2 N NaOH. It was shown to be L-2-[4-amino-4-carboxybutyl]iminol-5,5-cyclo tetramethylene-4-imidazolidinone (II in Fig. 1) by an independent synthesis. Reaction of S-methyl-5,5-cyclo tetramethylene-2-thiohydantoin and α-formyl-n-ornithine followed by acid hydrolysis yielded a compound identical in elementary composition and physical properties with the condensation product of Compound I and L-arginine. The melting point and optical activity of this compound are 240° with decomposition and [α]20 +18.2° ± 0.5° (c, 1, in 5 N HCl), respectively. It is stable in 6 N HCl at 110° for 20 hours.

The β chain of hemoglobin A (1), 40 mg, in 5.0 ml of 0.2 N NaOH was treated at 25° with 6.0 mg of Compound I, a 10-fold excess of reagent over arginyl residues. After 18 hours, the reaction mixture was dialyzed against water and lyophilized. Seventy-five per cent of the protein was recovered. 1,2-Cyclohexanedione-treated β chain and untreated β chain were hydrolyzed in 6 N HCl for 20 hours at 110°. Amino acid analyses of the hydrolysates demonstrated that arginine was absent in the treated sample and was replaced by a component which was indistinguishable from Compound II both by high voltage electrophoresis as shown in Fig. 2 and by column chromatography. No other amino acid was affected.

When tryptic digests of treated and untreated β chains were analyzed by a fingerprinting method (2), peptides joined by arginyl residues, namely, βTp3, βTp4, and βTp5 (3), were absent in the fingerprint of the treated hemoglobin. The other peptides of the untreated sample were present in the treated sample (Figs. 3 and 4); thus, tryptic hydrolysis of lysyl bonds was not blocked. No new peptide was observed; however, it is quite possible that a peptide as large as the unhydrolyzed sequence of βTp3-5 would not be satisfactorily resolved on a fingerprint.

The above results have shown that 1,2-cyclohexanedione reacts specifically with the guanido group of arginine, and that this reaction restricts tryptic hydrolysis of a protein to lysyl bonds. A similar result was obtained with benzil (4). The condensation product of benzil and L-arginine has also been found to be a substituted imidazolidinone, L-2-[4-amino-4-
Fig. 3. Fingerprint of tryptic digest of 1,2-cyclohexanedione-treated \(\beta\) chain of hemoglobin A. Descending chromatography in 1-butanol-acetic acid-pyridine-water (90:18:60:72). Electrophoresis for 2 hours at 35 volts per cm and 25° in pyridine-acetic acid-water (10:0.4:100), pH 6.5.
Fig. 4. Fingerprint of tryptic digest of \( \beta \) chain of hemoglobin A (control). For conditions see Fig. 3.


\(^2\) K. Toi and H. A. Itano, unpublished data.
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