possibly because of steric hindrance. HUGHES *et al.*[1] found that exhaustive reaction with O-methylisourea led to the reaction of 54–57 out of 64–68 amino groups, determined by Van Slyke amino nitrogen analysis. Partial reaction with GDMP occurs with the N-terminal amino groups of these proteins. About 60 % of the N-terminal aspartic acid of serum albumin reacted, while about 30 % of N-terminal leucine of lactoglobulin appeared to react.

More complete details and further studies on this reagent will be reported later.

I wish to express my thanks to Dr. DAVID B. SMITH for his helpful suggestions and advice throughout the work.

Division of Applied Biology, A. F. S. A. HABEEB*
National Research Council of Canada, Ottawa (Canada)

1 W. L. HUGHES, H. A. SAROFF AND A. L. CARNEY, *J. Am. Chem. Soc.*, 71 (1949) 2476.
2 F. L. SCOTT, D. G. O'DONOVAN AND J. REILLY, *J. Am. Chem. Soc.*, 75 (1953) 4053.
3 R. A. B. BANNARD, A. A. CASSELMAN, W. F. COCKBURN AND G. M. BROWN, *Can. J. Chem.*, 36 (1958) 1541.
4 V. J. HARDING AND R. M. MACLEAN, *J. Biol. Chem.*, 24 (1916) 503.
5 C. HUGGINS, D. F. TAPLEY AND E. V. JENSEN, *Nature*, 167 (1951) 592.
6 E. FRIEDMANN, D. H. MARRIAN AND I. SIMMON REUS, *Brit. J. Pharmacol.*, 4 (1949) 105.
7 L. R. MACDONNELL, R. B. SILVA AND E. R. FEENEY, *Arch. Biochem. Biophys.*, 32 (1951) 288.
8 H. FRAENKEL-CONRAT, J. I. HARRIS AND A. L. LEVY, in D. GLICK, *Methods of Biochemical Analysis*, Vol. II, New York 1955, p. 359.
9 A. F. S. A. HABEEB, *J. Pharm. and Pharmacol.*, 10 (1958) 591.
10 T. PETERS, A. G. LOGAN AND C. A. SANFORD, *Biochim. Biophys. Acta*, 30 (1958) 88.
11 R. R. PORTER AND F. SANGER, *Biochem. J.*, 42 (1948) 287.

Received March 31st, 1959

* National Research Council of Canada Postdoctorate Fellow.

The C-terminal amino acid of carboxypeptidase-A

Although it has been shown that CPase-A consists of a single polypeptide chain with N-terminal asparagine[2] and a molecular weight of 34,000[3,4], no information has yet been made concerning its C-terminal residue. The C-terminal amino acid of CPase-A has now been found to be asparagine both by the enzymic method, in which the native crystalline CPase-A was allowed to act upon the denatured enzyme, and by the catalytic-hydrazinolysis method.

CPase-A used in this experiment was a specimen crystallized from frozen bovine pancreas and recrystallized 6–10 times according to NEURATH's method[5], but including one DFP treatment. It was shown to be homogeneous ultracentrifugally and to yield aspartic acid (0.8–0.9 mole/mole) and serine (0.05–0.1 mole/mole) in the N-terminal determination by the DNP method, supporting the result obtained by THOMPSON[2]. The amino acid composition determined by the DNP procedure[6] was almost in agreement with that obtained by SMITH *et al.*[4].

The CPase-A was denatured by incubating at room temperature (23–25°) for 2–3 h with 0.2 % aq. sodium dodecyl sulfate (pH about 7) in the presence of 0.04 *M* *β*-phenyl propionate, a strong competitive inhibitor of the enzyme[7]. After dialysis

Abbreviations: CPase-A, carboxypeptidase-A (ANSON's[1]); CPase-B, basic carboxypeptidase; DFP, diisopropyl fluorophosphate; DNP, dinitrophenyl.

overnight in a refrigerator against 0.05 *M* Veronal buffer, pH 7.5, the solution of the denatured CPase-A (0.4 %) was incubated at 25° with crystalline CPase-A in the presence of 0.1 *M* NaCl and 10^{-3} *M* DFP (substrate: enzyme = 40:1, molar ratio). Aliquots removed from the digestion mixture at various time intervals were dinitrophenylated in aq. $NaHCO_3$ at 40° and analyzed for amino acids. The release of asparagine reached 1 mole/mole CPase-A after about 2-h incubation, followed by the release of valine (0.85 mole/mole), threonine (0.7), leucine (0.7), glutamic acid (0.5) and others. No change was found in the N-terminal residue of the protein before and after the digestion. When performic acid-oxidized CPase-A[8], hardly soluble in the buffer solution, was incubated at 25° for 20 h with CPase-A (substrate: enzyme = 50:1), an incomplete release of asparagine (about 0.4 mole/mole), threonine, valine, leucine and others could be observed. In contrast, no amino acid was liberated by the action of DFP-treated CPase-B (a fraction containing a strong CPase-B activity[9]) upon the oxidized CPase-A.

Catalytic hydrazinolysis at 60°[10] of crystalline CPase-A also revealed the C-terminal asparagine (about 1 mole/mole as its di-DNP-β-hydrazide after 24 h), but gave no appreciable amount of other amino acids. The C-terminal asparagine was successfully determined for the first time from the hydrazinolysate of protein[11], by modifying the extraction procedure[12] followed by identification on LEVY's two-dimensional paper chromatogram[6].

All these results show that CPase-A has asparagine in its C-terminal. Combining the result of THOMPSON with ours, it is concluded that the enzyme is composed of a single polypeptide chain, $Asp(NH_2)$. ---- (Glu, Leu, Thr, Val)·$Asp(NH_2)$. It may farther be inferred that trypsin is not responsible for the presence of the C-terminal residue after the activation of procarboxypeptidase-A, because no basic amino acid but asparagine was found in the C-terminus. This finding must be kept in mind in considering the course of the reaction process.

After completion of this manuscript, a paper by GRASSMANN AND RIEDEL[13] appeared stating that no C-terminus was found in CPase-A by the hydrazinolysis method. It is possible that these authors might have missed asparagine β-hydrazide from the C-terminus due to the aldehyde treatment.

TOSHIO ANDO
HIDEKO FUJIOKA
YASUHIRO KAWANISHI

Department of Biophysics and Biochemistry,
Faculty of Science, University of Tokyo (Japan)

1 M. L. ANSON, *J. Gen. Physiol.*, 20 (1937) 663.
2 E. O. P. THOMPSON, *Biochim. Biophys. Acta*, 10 (1953) 633.
3 E. L. SMITH, *Advances in Enzymol.*, 12 (1951) 191.
4 E. L. SMITH AND A. STOCKELL, *J. Biol. Chem.*, 207 (1954) 501.
5 H. NEURATH, *Methods in Enzymology*, Vol. II, Academic Press Inc., New York, 1955, p. 77.
6 A. L. LEVY, *Nature*, 174 (1954) 126.
7 E. ELKINS-KAUFMAN AND H. NEURATH, *J. Biol. Chem.*, 178 (1949) 645.
8 C. H. W. HIRS, *J. Biol. Chem.*, 219 (1956) 611.
9 T. ANDO, T. TOBITA AND M. YAMASAKI, *J. Biochem. (Tokyo)*, 45 (1958) 285.
10 J. H. BRADBURY, *Nature*, 178 (1956) 912.
11 T. ANDO AND Y. KAWANISHI, in preparation.
12 S. AKABORI, K. OHNO, T. IKENAKA, Y. OKADA, H. HANAFUSA, I. HARUNA, A. TSUGITA, K. SUGAE AND T. MATSUSHIMA, *Bull. Chem. Soc., Japan*, 29 (1956) 507.
13 W. GRASSMANN AND A. RIEDEL, *Z. physiol. Chem.*, 313 (1958) 227.

Received March 23rd, 1959