sequences Ala-Gly-Ser, Ala-Gly-Gly and Ser-Gly-Ala. These preliminary experiments indicate that similar degradation procedures, carried out on a larger scale, should yield peptides from blood-group-specific glycoproteins suitable for detailed sequence analysis.

- Abdel-Akher, M., Hamilton, J. K., Montgomery, R. & Smith, F. (1952). *J. Am. chem. Soc.* **74**, 4970.
 Donald, A. S. R., Creeth, J. M., Morgan, W. T. J. & Watkins, W. M. (1969). *Biochem. J.* **115**, 125.
 Gray, W. R. (1967). In *Methods in Enzymology*, vol. 11, p. 469. Ed. by Hirs, C. H. W. New York and London: Academic Press.

Isolation, Purification and Characterization of a 0.6S γ_2 -Globulin from Normal Human Plasma

By R. B. NIMBERG and K. SCHMID. (*Department of Biochemistry, Boston University School of Medicine, Boston University Medical Center, Boston, Mass. 02118, U.S.A.*)

Of the large number of proteins that have been isolated from normal human plasma, most are non-dialyzeable and of relatively high molecular weight. In contrast, in this communication, we now report the isolation of a diffusible low-molecular-weight protein fraction and the purification and partial characterization of one of its constituents.

Pooled normal human plasma was fractionated by Cohn method 6 (Cohn *et al.* 1946). The proteins present in the supernatant solution of fraction V were rendered insoluble by a previously described procedure (Bürgi & Schmid, 1961), dissolved at -5°C with EDTA and then separated on a DEAE-cellulose column at pH 5.5 into two fractions (Iwasaki & Schmid, 1967). The main fraction was then separated into Zn^{2+} -soluble and Zn^{2+} -precipitable proteins (Schmid, 1953), and the precipitate was subsequently solubilized and further purified by column chromatography on DEAE-cellulose at pH 5.4. The bulk of the proteins was retained, and the effluent fraction contained γ_2 -, γ_1 - and β -globulins as judged by paper electrophoresis at pH 8.6. On ultracentrifugal analysis this mixture revealed a single symmetrical refractive-index gradient, sedimenting with a coefficient of 0.6S. Purification of one of these components, a γ_2 -globulin, was subsequently achieved by ion-exchange chromatography on TEAE- (triethylaminoethyl-) and CM-cellulose. Homogeneity of this protein was established by polyacrylamide-gel electrophoresis at pH 8.3 and 4.5 and N- and C-terminal amino acid analyses.

Characterization of the homogeneous 0.6S γ_2 -globulin indicated that this protein was devoid

of neutral hexoses and sialic acid. The molecular weight determined by the sedimentation-equilibrium technique of Yphantis (1964) was found to be 5100. With regard to its conformation, preliminary analysis of circular-dichroism spectra suggest that this protein possesses a small percentage of α -helix and contains considerable amounts of β -structure and random coil.

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- Bürgi, W. & Schmid, K. (1961). *J. biol. Chem.* **236**, 1066.
 Cohn, E. J., Strong, L. E., Hughes, W. L., jun., Mulford, D. J., Ashworth, J. N., Melin, M. & Taylor, H. L. (1946). *J. Am. chem. Soc.* **68**, 459.
 Iwasaki, T. & Schmid, K. (1967). *J. biol. Chem.* **242**, 5247.
 Schmid, K. (1953). *J. Am. chem. Soc.* **75**, 60.
 Yphantis, D. A. (1964). *Biochemistry, Easton*, **3**, 297.

The Complete Hydrolysis of Peptides by a Mixture of Sepharose-Bound Peptidases

By H. P. J. BENNETT, D. F. ELLIOTT, P. J. LOWRY and C. McMARTIN. (*Research Division, CIBA Laboratories, Horsham, Sussex RH12 4AB, U.K.*)

Hill & Schmidt (1962) reported that incubation of polypeptides with a series of peptidases resulted in quantitative non-destructive release of amino acids. The method offers advantages over acid hydrolysis where destruction of serine, tyrosine, tryptophan, asparagine and glutamine occur.

To simplify the procedure we used a mixture of prolidase, aminopeptidase-M, trypsin and chymotrypsin bound to Sepharose (Hurwitz, Dietrich & Sela, 1970). Prolidase was prepared by using the first two steps of the method of Davis & Smith (1957). All the bound enzymes were found to be stable.

The bound peptidases (200 mg each of Sepharose-trypsin, Sepharose-chymotrypsin and Sepharose-prolidase and 400 mg of Sepharose-aminopeptidase-M) were stored as a mixture in 10 ml of tris buffer (50 mM, pH 7.4, 0.03% toluene, 0.5% thiodiglycol and 10 mM- Mn^{2+}). Incubations were performed with a 50–200 μl sample of the mixture (1 ml/mg of substrate) in a stoppered polystyrene tube. The mixtures were agitated gently for 24 h at 25°C . Blank amino acid contents when the slurry was incubated alone were equivalent to less than 3% of a single residue and were subtracted from the amino acid values obtained from substrates. Repeat digestions of synthetic β 1–24 adrenocorticotrophin (Synacthen; CIBA, Basle, Switzerland) revealed theoretical amino acid molar