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.

This investigation was supported by a Postdoctoral Fellowship (5F02DE44743) and a Research Grant (GM-10374) from the National Institute of Health, U.S. Public Health Service.

- 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

proportions, with a standard deviation per residue of less than 5%.

Amino acids were also released quantitatively from bradykinin and [5-valine]-angiotensin II amide.

Incubations of two D-residue analogues of [5-valine]-angiotensin II amide ([6-D-histidine]- and [2-D-arginine]-angiotensin II amide) showed that bonds adjacent to D-amino acid residues were not cleaved.

Enzyme digestion was applied to Synacthen that had been partially racemized by alkali treatment (Lee & Buettner-Janusch, 1963). The results indicated where extensive racemization had probably occurred.

Incubation of performic acid-oxidized ribonuclease showed that aspartic acid and glutamic acid could be detected separately from asparagine and glutamine. Glutamine and methionine sulphone were not released quantitatively, and the amounts of these amino acids did not increase when the time of incubation was increased.

Hydrolysis by bound peptidases might be a useful method both as a purity check for synthetic peptides and in the elucidation of peptide and protein amino acid sequences.

The authors thank Mr B. Evans for performing the amino acid analyses, Dr R. Wade for the bradykinin and Dr B. Riniker for the angiotensins.

Davis, N. C. & Smith, E. L. (1957). *J. biol. Chem.* **224**, 261.
Hill, R. L. & Schmidt, W. R. (1962). *J. biol. Chem.* **237**, 389.

Hurwitz, E., Dietrich, F. M. & Sela, M. (1970). *Eur. J. Biochem.* **17**, 273.

Lee, T. H. & Buettner-Janusch, V. (1963). *J. biol. Chem.* **238**, 2012.

Model of an Insulin Dimer and Tetramer, and a Hypothetical Proinsulin

By IVOR SMITH. (*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR, U.K.*)

Revised X-ray co-ordinates for insulin giving data for both the main chain and R-group atoms have become available (Blundell *et al.* 1971), and from these a number of atomic models of the monomer have been built. However, as the molecule crystallizes as a hexamer composed of three identical dimer pairs, I have constructed a Biobits model (Smith & Smith, 1970; Smith, Smith & Roberts, 1970) illustrating various points that will not be seen from the atomic monomer model.

First, a complete insulin dimer showing both α - and β -chains together with all R groups was constructed. Secondly, a single-backbone-only mono-

mer was built on each side of the complete dimer, thus enabling a tetramer to be examined from all sides. The two zinc atoms are included so that a complete idea of the hexamer can be visualized.

The model is extremely lightweight and stable so that it can be turned and examined from all directions. Possible peptide chain interactions can be considered as well as R-group interactions and, because each R group is represented by a single bit, such groups can be readily inserted or removed without disturbing the basic molecular structure. It is also possible to insert the proinsulin intermediate peptide and to hypothesize on the three-dimensional structure of this molecule, on the different immunological and other observed reactions between the two etc.

Blundell, T. L., Cutfield, J. F., Cutfield, S. M., Hodgkin, D., Dodson, E. J., Mercola, D. A. & Vijayan, M. (1971). *Nature, London.*, **231**, 506.

Smith, I. & Smith, M. J. (1970). *Br. J. Hosp. Med.* p. 666.

Smith, I., Smith, M. J. & Roberts, L. (1970). *J. Chem. Educ.* **47**, 302.

Antimicrobial Proteins in Bovine Neutrophils

By J. BROWNLIE. (*Agricultural Research Council Institute for Research on Animal Diseases, Compton, Newbury, Berks., U.K.*)

Milk of cows with mild mastitis has greater antimicrobial activity than has normal milk (Derbyshire, 1964). The phagocytic activity of the cellular factor has been described (Elek, 1959), but there has been less definition of the antimicrobial humoral factors in mastitis milk. One of the humoral factors described is a basic protein fraction with antimicrobial activity isolated from the cells in bulk milk samples (Hibbitt, Brownlie & Cole, 1971). It was stable to heating at 70°C for 30 min. The present work indicates that an acid-extractable fraction is obtainable from the cells of mastitis milk, consisting of 95–99% neutrophils, with activity against streptococci and both coagulase-positive and coagulase-negative staphylococci.

A sterile mild mastitis was induced in the cow by infusion of 1 μ g of an *Escherichia coli* lipopolysaccharide endotoxin through the teat canal and the milk was collected 16 h later. Cells were removed from the milk by centrifuging at 400 g_{av} and washed three times in a cold iso-osmotic saline. The cells were homogenized in ice-cold 0.2 M-hydrochloric acid in a Waring Blendor at full speed for 2 min, then centrifuged at 20 000 g_{av} for 10 min. The clear supernatant was dialysed against five changes of double-glass-distilled water, and any