$Na_2S_2O_4$ aktivierbare Glyzerinsäure-2.3-diphosphatase (2.3-PGase) verantwortlich. Das Enzym wurde aus Azeton-Trockenpulver von Menschen- und Schweine-Erythrozyten durch Fraktionierung mit Alkohol und Ammoniumsulfat teilweise gereinigt. Seine Wirkung ist spezifisch auf 2.3-PGS, 3-PGS wird nicht gespalten. Es unterscheidet sich bezüglich seiner Eigenschaften von der, von RAPOPORT und Mitarbeitern[2,3] aus Muskel hergestellten 2.3-PGase. Für die Aktivität des Erythrozyten-Enzyms sind ausser $NaHSO_3$ bzw. $Na_2S_2O_4$, einwertige Kationen (K^+, Na^+, NH_4^+) in isotonischer Konzentration unbedingt erforderlich. Das Enzym wird durch Hg^{++} Ionen, im Gegensatz zur Muskel-2.3-PGase, nicht aktiviert.

Eine ausführliche Veröffentlichung der Experimente, samt einer Diskussion ob 2.3-PGS ein obligates Zwischenprodukt der Hämoglykolyse sei, erscheint in *Acta Physiol. Hung.*

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Isolation of DNP-peptides from DNP-polyvalyl-proteins*

The difficulty in obtaining significant yields of protein fragments larger than dipeptides by acid hydrolysis has been emphasized by SANGER, and a variety of milder hydrolytic conditions have been used for the isolation of larger peptides[1]. During the course of end group studies of polyvalyl-proteins, we have found that peptides containing as many as 9 amino acids from the protein chain, in addition to the added valine, remained after hydrolysis under conditions usually employed for the isolation of DNP-amino acids**. This apparent stabilization of sections of the polypeptide chains by valine peptides should offer advantages in studying protein structure, since the products of the strong acid hydrolysis would be a relatively simple mixture of peptides and free amino acids.

The polyvalyl-proteins were prepared by the reaction of the proteins with N-carboxy-DL-valine anhydride[2]. These were reacted with 1,2,4-fluorodinitrobenzene[3] and subsequently hydrolyzed with 5.7*N* HCl at 105° C in a sealed tube for 16 hours. The peptides were isolated by extraction of the hydrolysate with ethyl acetate***, followed by separation on silicic acid columns, using water-saturated $CHCl_3$ as the mobile phase[4]. A further check of purity of the DNP-peptides obtained was determined by two-dimensional paper chromatography using phenol-*iso*amyl alcohol-water (1:1:1) in one direction and phthalate buffer (pH 6.0) in the other, in addition to the column chromatography using two different eluting solvents. Under identical experimental conditions no DNP-peptides were found when the DNP derivatives of unmodified, poly-L-phenylalanyl- or polyglycyl-proteins were hydrolyzed.

Since its amino acid sequence is known[5,6], insulin offered a convenient model protein for study. From 19 μM of DNP-polyvalyl-insulin, 7 μM of ε-DNP-lysine, two peptides (3 μM and 8 μM) which differed only in the amount of valine present, and 25 μM of DNP-valine, were obtained. Hydrolysis of the peptides in 5.7*N* HCl at 105° for 96 hours liberated the following amino acids in addition to from 5 to 10 moles of valine[7,8]: Lys, Phe, Gly, Ala, Thr, Glu, Tyr, Pro, and Arg. Glycine was N-terminal[4] and alanine C-terminal[9] and the ratio of phenylalanine to lysine was two[10]. The peptide is therefore from the C-terminal end of the phenylalanine chain, which contains the only lysine and proline in the molecule. The specificity of the reaction is such that within the limits of the methods used, only amino groups react[11]. The results show that over 90% of the lysine is accounted for either in the peptides or as ε-DNP-lysine, but the recovery of the DNP-valine is not sufficient to account for all of the amino groups. No other DNP-amino acids were found, nor were any peptides from the N-terminal ends isolated.

DNP-peptides of varying size, but all containing lysine, have been obtained from chymotrypsin, trypsin, lysozyme and bovine plasma albumin when similar experiments were carried out. The yields of the peptides, based upon spectrophotometric determination of the DNP-valine

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** Abbreviations: DNP = Dinitrophenyl; amino acids according to E. BRAND AND J.T. EDSALL, *Ann. Rev. Biochem.*, 16 (1947) 223.

*** The aqueous may also contain peptides, and has not as yet been investigated.

present in the DNP-peptides, have varied from about 40–70%. Detailed studies to determine the maximum yields, as well as variations in peptide size, have not been completed.

The stability of peptides containing valine to acid hydrolysis is well known, and is attributed to steric hindrance[12]. The manner in which the valine peptides provide stabilization of peptide bonds surrounding lysine might involve other factors as well. The results obtained suggest that compounds other than valine peptides might function similarly, and that this approach to the problem of degradation of proteins for structural studies may prove advantageous. Studies with *iso*leucine peptides[13], and with compounds which exhibit a high degree of steric hindrance and are not normal constituents of proteins, are in progress.

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Enzymic degradation of heparin

Heparin is a mucopolysaccharide reported to contain equimolar amounts of glucosamine and glucuronic acid with one to three sulfate moieties per disaccharide unit[1]. One sulfate group is bound in an amide linkage to the amino group of glucosamine; the others are esterified to sugar alcohol groups. The isolation and adaptation of a bacterium which can utilize heparin as its sole carbon, nitrogen and sulfur source were described in a recent communication from this laboratory[2]. It is now desired to present data on the degradation of heparin by extracts of acetone powders of the adapted bacteria.

Bacteria were grown and adapted as previously described[2]. The adapted cells were then blended in acetone (—20° C), filtered, air-dried at room temperature and stored in a vacuum desiccator at —15° C. Heparin degradation was routinely followed by either the decrease of metachromasia with azure A or the increase in reducing groups; essentially identical results were obtained by both assays. The rate of degradation of heparin was found to be directly proportional to the concentration of extract used; 0.5 mg of protein catalyzed the complete degradation of 1 mg of sodium heparin in approximately one hour at 24° C. The "heparinase" activity was completely stable to dialysis against distilled water. The activity was completely destroyed upon heating at 40° C for 5 minutes and the preparation was completely inactive when assayed in the presence of any salt in a concentration of 0.1 to 0.2 *M*. The optimum pH for "heparinase" action was found to be between 7 and 7.5.

Since glycylglycine buffer extracts of the acetone powder were not as active as the phosphate buffer extracts, we suspected that the reaction sequence might involve a phosphorylysis. The data summarized in Table I, however, seem to have eliminated this possibility. Although the "heparinase" activity was stimulated by phosphate and arsenate, citrate and at least two cations —magnesium and ammonium—were also active. Pyrophosphate and ethylenediaminetetra-acetate (EDTA) completely inhibited activity regardless of whether anions or cations were used for activation. The EDTA-inhibited preparation could be reactivated, after dialysis against water, by either phosphate, magnesium or ammonium ions.

The "heparinase" activity of these extracts is the result of at least three enzymes which act in a yet undetermined sequence on heparin and degradative products thereof (Table II). The increase in reducing groups indicates the presence of a glycosidase; the increase in amino sugar