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

denotes the action of an amino sulfatase (and also indicates that the reducing groups of glucosamine have been freed); and the high ratio of periodate consumption to reducing groups indicates that an alcohol sulfatase has split the ester sulfate bonds. We are currently engaged in the isolation and characterization of the degradation products and the separation of the enzymes involved.

TABLE I

ACTIVATION OF "HEPARINASE" BY CERTAIN IONS

Addition	D_{490}/*hour*
None	0.07
Phosphate, 0.01 *M*	0.17
Arsenate, 0.01 *M*	0.17
Citrate, 0.01 *M*	0.15
Mg^{++}, 0.005 *M*	0.17
NH_4^+, 0.05 *M*	0.20

All vessels contained 0.3 ml of a water extract of the acetone powder (40 mg/ml), glycylglycine buffer, pH 7.5 and 0.6 mg of sodium heparin in a total volume of 0.6 ml. The phosphate, arsenate and citrate solutions were adjusted to pH 7.5 before addition and the total buffer molarity in all vessels was 0.025. The vessels were incubated at 24° and duplicate aliquots of 0.02 ml removed for determination of metachromasia with azure A[3].

TABLE II

ACTION OF "HEPARINASE" ON HEPARIN

	0	*30 min*	*60 min*
Metachromasia (D_{490})	0.30	0.24	0.06
Reducing groups (μM)	0	1.4	2.8
Amino sugar (μM)	0	0.8	2.5
Periodate consumption (μM)	0	—	16

The incubation vessel contained 2.5 ml of acetone powder extract (10 mg of acetone powder/ml of 0.025 *M* phosphate buffer, pH 8.0) and 4 mg of sodium heparin in a total volume of 4 ml of 0.025 *M* phosphate buffer, pH 7.5. The vessel was incubated at 24° and duplicate aliquots removed and analyzed as indicated. Glucosamine was used as the standard for the amino sugar determination[4] and an equimolar mixture of glucosamine and glucuronic acid for the reducing group determinations[5]. Periodate consumption was measured by a spectrophotometric method[6].

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