Acid hydrolysates of a variety of bovine trypsin preparations have been investigated. Twice-recrystallized trypsin from Worthington Biochemicals and from Mann Chemicals, a five-times recrystallized preparation from Worthington Biochemicals, a three-times recrystallized preparation made in our laboratory and a three-times recrystallized preparation of diisopropylphosphoryl-trypsin were included in these studies. The presence of hydroxylysine, demonstrated in hydrolysates of all of these preparations, suggests that this amino acid is a constituent of the trypsin molecule.

In a previous amino acid analysis of trypsin reported from this laboratory[11] the hydroxylysine was undetected, apparently because the basic amino acids were determined by use of the 15-cm column[3] for basic amino acids instead of the 50-cm column[3] used in this work.

In one analysis of an acid hydrolysate of bovine chymotrypsin a total of 0.6 mole of hydroxylysine and allohydroxylysine per mole of protein was found.

The details of this work will be published elsewhere.

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A nucleotide enzyme complex associated with fowl leukemia virus

The virus particles that can be isolated from blood plasma in the case of fowl myeloblastic leukemia possess a high magnesium-stimulated ATPase activity[1–3]. ADP has been claimed to act as a competitive inhibitor in the hydrolysis of ATP[4].

A closer study of the viral ATPase activity reveals properties, some of which indicate a complex of phosphorylating enzymes. If activated by Mg^{++}, two pH optima can be distinguished (pH 7.0 and 8.5). Besides Mg^{++}, also other metal ions activate

Abbreviations: NTP, ATP, UTP, CTP and GTP for the 5′-triphosphates; NDP, ADP, UDP, CDP and GDP for the 5′-diphosphates; NMP, AMP, UMP, CMP and GMP for the 5′-monophosphates of nucleosides, adenosine, uridine, cytosine and guanosine; P_i, inorganic orthophosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

the enzyme complex in the proportions: Mg^{++} 1.0, Cd^{++} 0.5, Fe^{++} 0.5, Zn^{++} 0.25 and Cu^{++} 0.25.

The enzyme complex splits inorganic orthophosphate from nucleoside diphosphates at pH 7.0 in the sequence of activities: ADP < UDP < CDP < GDP, and at pH 8.5 UDP < ADP = CDP < GDP. The quantitative analysis of these reactions shows that at least two events take place: NDP $\rightleftharpoons$ NMP + P_i (ref. 5, 6); and n NDP $\rightleftharpoons$ (NMP)n + n P_i (ref. 8). To these reactions can be added the ATPase activity: ATP $\rightarrow$ ADP + P_i, when ATP is used as the substrate.

Thus, in addition to a high triphosphatase activity, a polynucleotide–phosphorylase (polyase) activity is present, which reaction runs most efficiently if GDP is used as the substrate (GDP $\gg$ CDP > UDP > ADP, see Table I).

TABLE I

Enzyme reactions with fowl leukemia virus isolated from blood plasma[2,14]. Viral enzyme concentrations, 2.0 and 8.0 μg N/ml reaction mixture[15]. Incubation medium: 0.25 *M* sucrose and 0.04 *M* Tris-buffer. P_i estimation according to ref. 16. The analyses of the polyase, nucleoside diphosphatase and myokinase activities were made in principle as described by BEERS[17]. Chromatography of the reaction mixtures was performed on Whatman paper with the isobutyric acid–NH_3–EDTA mixture as solvent. The spots were eluted with 0.1 *N* NH_4OH for spectrophotometric determinations.

Substrate	*μmoles Pi/mg N/30 min Substrate, 2.5 mM; Mg^{++}, 3.5 mM; sucrose-Tris; 37°*		*(NMP)n as μmoles NMP/mgN in 30 min*	*μmoles Pi/mg N in 30 min*
	pH 7.0	*pH 8.5*	*Substrate, 6.1 mM; Mg^{++}, 1.5 mM; Tris, pH 8.5; 37°*	
ADP	36.6	40.6 –	4.61	38.0
UDP	40.0	33.3	7.32	29.4
CDP	48.4	41.3	11.95	40.0
GDP	58.9	53.9	65.0	58.0
ATP	364.0	408.0	> ADP	445.0

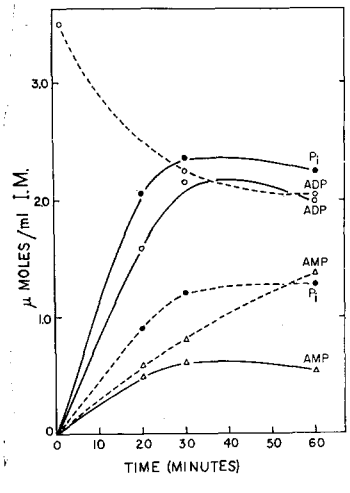

Fig. 1. Viral enzyme complex incubated with 2.5 m*M* ATP (——) or 4.0 m*M* ADP (– – –). Incubation medium (I.M.): 0.25 *M* sucrose; 0.04 *M* Tris, pH 8.5; 1.5 m*M* Mg^{++}; 37°. ADP ○, AMP △, P_i ●.

The synthetisizing polyase activities are associated with a diphosphatase and possibly also a myokinase-like activity according to the terminology of GIBSON *et al.*[7]. This conclusion is drawn from the decrease of AMP during the later part of the incubation (> 30 min, ATP as substrate, see Fig. 1), as well as from the simultaneous decrease of P_i in the same period. The sequence of activities when different nucleoside diphosphates are incubated is similar to that of the polyase: GDP > CDP > ADP > UDP.

If the activities of the enzyme complex are expressed in specific activities (units/mg N/min), the figures will roughly correspond to the specific activities measured in

purified enzymes from other sources, *e.g.* the adenylate kinase, nucleoside diphosphatase from liver[6,7]. The six to ten times higher activity of the GMP phosphorylase in comparison with the other polyase activities is noteworthy from the point of view of specificity and heterogeneity[8–10] of the polyase enzymes and also of the tumor polynucleotides[11].

Finally, it should be mentioned that ATPase activity with characteristics of the electron-transport chain is also present. Perhaps the viral enzyme complex represents a reminiscence of those parts in the host cell which have participated in the virus synthesis[12]. The view[13] "that viruses do not act only as cell and tissue destroyers but as parts of normal animal cells" conforms with this hypothesis.

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Interference with adenine and histidine metabolism of microorganisms by aminotriazole

3-Amino-1,2,4-triazole (aminotriazole), a heterocyclic nitrogen compound used extensively in agriculture as a weed killer, bears a close structural relationship to 4-aminoimidazole. 4-Aminoimidazole is recognized as an intermediate in purine degradation in *Clostridium cylindrosporum*[1] and 4-aminoimidazole ribonucleotide is an intermediate in purine biosynthesis[2]. From these considerations, it might be