

ENZYMATIC PROPERTIES OF STAPHYLOCOAGULASE-HUMAN PROTHROMBIN COMPLEX (STAPHYLOTHROMBIN).

S. Kawabata, T. Morita, H. Igarashi¹ and S. Iwanaga.
Department of Biology, Faculty of Science, Kyushu University
Fukuoka, and ¹Tokyo Metropolitan Research Laboratory of
Public Health, Tokyo, Japan

Staphylocoagulase(SC) is an extracellular protein produced by *Staphylococcus aureus*. This protein is known to coagulate several mammalian plasmas, by forming an active molecular complex with prothrombin. However, there is not enough knowledge of the molecular mechanism of prothrombin activation by SC. The present work was undertaken to elucidate the enzymatic properties of SC-human prothrombin(HP) complex. A highly purified SC was prepared from a culture filtrate of *Staphylococcus aureus*, strain st-213, by a bovine prothrombin-Sepharose affinity column developed in our laboratory. This SC could activate HP but not bovine prothrombin. A SC-HP complex formed with the molar ratio of 1 to 1 had a strong amidase activity towards α -thrombin fluorogenic substrate, Boc-Val-Pro-Arg-4-methylcoumaryl-7-amide. The activity of the complex was stable at 0°C for at least 8 hrs, while the activity was rapidly decreased with incubation at 37°C. SDS-polyacrylamide gel electrophoretic analysis on the complex formed at 37°C indicated not only a proteolysis of SC portion to small derivatives, but also the fragmentation of HP portion to prethrombin 1 and profragment 1. This proteolytic fragmentation seemed to be due to a self-catalytic reaction of the SC-HP complex, since no endogeneous proteolytic activities were detected in each preparation of SC and HP. Bovine prothrombin, prethrombin 1 and prethrombin 2 inhibited the complex formation between SC and HP, but bovine profragment 1 and 2 had no effect on the complex formation. These results indicate that a high affinity site of HP to SC must be located in the prethrombin 2 region. Like α -thrombin, the amidase activity of SC-HP complex was competitively inhibited with benzamidine ($K_i=1.2 \times 10^{-4}M$). However, both clotting and amidase activities of the complex could not be inhibited by 4 molar excess of hirudin or antithrombin III in the presence of heparin.

THE AMINO ACID SEQUENCE STUDIES ON *Limulus polyphemus* COAGULOGEN. T. MIYATA, M. UMEZU and S. IWANAGA. Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan

Coagulogen, which is a clottable protein found in the amoebocyte lysate of *Limulus polyphemus*, consists of a single basic polypeptide chain having the total of 174 amino acid residues. Upon gelation of this protein, a large peptide, named peptide C, which has 28 amino acid residues, was released, and the resulting gel protein consisted of A and B chains, bridged by two disulfide linkages. This work is to determine the whole sequence of B chain and to compare with the primary structure of *Tachypleus tridentatus* (Japanese horseshoe crab) coagulogen previously established. The *Limulus* B chain was digested with trypsin and the resulting fragments were separated into 17 major peptides. For the sequence determination, manual Edman degradation was employed and resulting PTH amino acids were identified by HPLC. These analyses established the sequence of the isolated tryptic peptides, ending with the COOH-terminal serine, and the following whole sequence of *Limulus* coagulogen was presumed, comparing with that of *Tachypleus* coagulogen.

Tachypleus: NH₂-A-D-T-N-A-P-I-C-L-C-D-E-P-G-V-L-G-R-T-Q-I-V-S-Q-E-T-K-D-K-I-E-E-A-V-Q-A-I-T-B-K-D-E-I-S-G-R-G-F-S-I-F-G-H-H-P-V-F-R-E-C-G-K-Y-E-C-R-T-V-R-P-E-H-S-R-C-Y-N-F-P-P-F-T-H-G-P-A-F-K-E-C-G-K-Y-E-C-R-T-V-T-S-E-D-S-R-C-Y-N-F-F-P-F-S-H-F-K-L-E-C-P-V-S-T-R-D-C-E-P-V-F-C-Y-T-V-A-G-E-F-R-V-I-V-Q-H-F-H-P-Z-C-P-T-S-T-S-B-C-Z-P-V-L-S-Y-T-V-A-G-Z-F-R-I-I-V-Q-A-P-R-A-G-F-R-Q-C-V-W-Q-H-K-C-R-F-G-S-N-S-C-G-Y-N-G-R-C-T-Q-A-P-K-A-G-F-R-D-L-E(W)V.H.K.C.R.A-Y-G-S-N-F-C-Q-S-Z-R.C-T-Q-Q-R-S-V-V-R-L-V-T-Y-N-L-E-K-D-G-F-L-C-E-S-F-R-T-C-C-G-C-P-C-Q-R-S-V-V-R-L-V-T-Y-D-L-E-K-G-V-F-F-C-E-N-V-R-T-C-C-G-C-P-C-R-S-F-COOH
R.S-COOH