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ISOLATION AND CHARACTERIZATION OF MICROBIAL IMMUNOGLOBULIN A PR--ETC(U)
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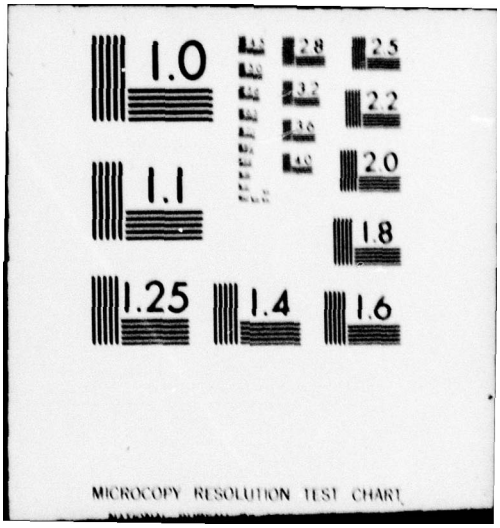
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(10) Andrew G. Plaut, M. D.

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FINAL REPORT

Contact No. DAMD 17-74-C-4022

The following is a scientific report covering the period January 1, 1974 to December 31, 1976 under the contract DAMD 17-74-C-4022.

In general, this research involved the enzyme IgA protease, an extracellular, neutral proteolytic enzyme isolated from culture filtrates of Neisseria gonorrhoeae, N. meningitidis, and the oral streptococcus, S. sanguis.

The research period began with attempts to identify an organism which could be counted upon to produce the enzyme in sufficient yield to allow reliable growth and relative ease of purification. We settled on S. sanguis of Lancefield Group H, an agent isolated years ago from a patient with bacterial endocarditis, and now sold by the American Type Culture Collection as Streptococcal species #10556. We were able to identify and utilize optimal growth conditions for this organism to yield large amounts of IgA protease. A purification scheme was developed involving stepwise:

1) Growth of S. sanguis #10556 in Todd Hewitt broth culture (conditions are detailed in our Progress Report for the year 1974).
2) Precipitation of IgA protease activity with ammonium sulfate treatment of bacteria - free filtrates.
3) Dissolution of precipitate containing the enzyme, and chromatography of the solution on polyacrylamide molecular seive columns. Identification was by enzyme assay as outlined below.
4) Rechromatography of enzyme in polyacrylamide or polysaccharide molecular seive columns of larger pore size than used in step 3.

The protein purification scheme yielded a preparation of enzyme which allowed kinetic studies of its activity, and the analysis of the primary structure of large fragments of IgA which resulted from enzyme activity on this molecule.

After isolation of IgA protease we were able to show that it was truly specific for human IgA immunoglobulin as substrate, and that it was inactive on all other protein substrates examined. Further, only proteins of the IgA1 subclass were susceptible, IgA2 human myeloma paraproteins being totally resistant.

The insusceptibility of IgA2 subclass proteins was explained by rather surprising and provocative amino acid sequence data of the Fc α fragment derived from IgA1 myeloma protein. A limited amino-terminal sequence of an S. sanguis - derived human Fc α fragment was as follows:

-thr-pro-ser-pro-ser-thr-pro-pro-thr-pro-ser-pro-ser. This localized the enzyme susceptible peptide bond to a pro-thr-in the IgA1 hinge region. IgA2 proteins were then understandably insusceptible, owing to their lack of the initial threonyl residue in this bond. The absence of the threonyl arises because IgA2 proteins have a 13 amino acid deletion (in the enzyme-accessible hinge peptide) which begins with the very threonyl residue involved in the peptide bond cleaved by IgA protease.

In conjunction with the late Malcolm Artenstein at WRAIR, we were able to show that the two pathogens Neisseria gonorrhoeae and N. meningitidis produce enzymes similar in specificity to streptococcal IgA protease. Analysis of the primary sequence of the resultant Fc α fragment generated by N. gonorrhoeae IgA protease revealed the following sequence:

-thr-pro-ser-pro-ser-cys-cys-his-pro-arg-

When aligned with the known sequence of the IgA1 hinge region, it became apparent that gonococcal IgA protease was cleaving the protein at a pro-thr bond differing in location from that cleaved by the streptococcal enzyme. The gonococcal-susceptible bond is in one of the halves of a duplicated hinge region segment in the IgA1 hinge region, while the streptococcal-susceptible prolyl-threonyl bond lay in the other. These relationships are best appreciated in Figure 2 of the publication by Plaut et al. (Science 190, 1103, 1975). The sites of enzymatic

attack by both enzymes on the IgA1 protein are both absent in IgA2 proteins due to the aforementioned sequence deletion in the hinge which characterizes this subclass.

The discovery of these enzymes raised the question of their possible role in pathogenesis of Neisserial infections. Subsequent work supported by renewals of the Contract involved several areas including the development of an assay method for the IgA proteases, the purification of the newly identified gonococcal IgA protease, and the establishment of methods for the quantitation of the two human IgA subclasses in secretions.

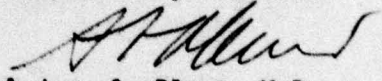
Purification of the gonococcal enzyme generally proceeded along lines similar to that of S. sanguis as outlined above. Once again, a preparation sufficiently pure to allow for primary sequence analysis of IgA fragment generated by the enzyme was achieved. One technical problem is the difficulty of growing N. gonorrhoeae in liquid culture in sufficient amounts to obtain high enzyme yields.

Assay of the IgA proteases is rather cumbersome and technically taxing because human IgA proteins are the obligate substrate. The procedure we attempted for assay were numerous, most involving electrophoresis of cleavage products of IgA on various supports. The most useful method, eventually adopted, was separation of ^{125}I labelled cleavage products of human IgA paraproteins by cellulose acetate electrophoresis. The Fab α fragment was removed by cutting the membrane and amount of Fab formed per unit time by gamma counting was then determined. This method has allowed us to study characteristics of the S. sanguis enzyme with respect to its kinetics and optimum conditions for activity. In the case of N. gonorrhoeae IgA protease, the cleavage product of IgA separate somewhat less favorably on electrophoresis, and pre-treatment of the substrate protein with neuraminidase to remove sialic acid residues is needed to correct this problem. At the time the Contract was terminated we were preparing to study the characteristics of gonococcal IgA protease with this new technique.

Finally, we had established a radioimmunoassay procedure for the quantitation of the two subclasses of IgA in secretions. This was an arduous task, involving preparation of antisera specific for the two subclasses, repeated radiolabelling of highly purified human IgA1 and IgA2 paraproteins, and an extensive analysis of the potential problem of secretory component in obstructing the antigenicity of alpha chain in these reactions. At the time of termination of the contract, we were planning to use the assay to study human urogenital secretions for their IgA subclass content. This work was undertaken to study the critical question as to whether susceptibility to gonorrhea is correlated with a certain IgA1/IgA2 ratio in secretions. The documentation of the RIA technique was submitted in the final report for the year 1976.

The undersigned would like to thank the U.S.A. R & D Command for providing funds to do this interesting work on the microbial IgA proteases.

Respectfully submitted



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PUBLICATIONS ARISING FROM THIS RESEARCH

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