COMPOSITION AND MOLECULAR SHAPE OF CHYMOTRYPSIN-NUCLEIC ACID COMPLEXES

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It has been found that the hydrolysis of methylhippurate (0.01 M) by chymotrypsin (ChT), determined by the pH-stat technique in the absence of added salt at pH 7.5 and 30°, is strongly inhibited by various polymers such as denatured proteins (Hofstee, 1960 a), carboxymethyl cellulose and nucleic acids (Hofstee, 1960 b). In all cases the inhibition was prevented by KCl in concentrations of the order of 0.1 M. Only "highly polymerized" DNA (Worthington) showed irreversible binding as indicated by sharp breaks in plots of inhibition (ordinate) versus DNA concentration (abscissa). The horizontal part of such a plot indicated a maximal inhibition of about 80 percent, and from the intercept of the two linear portions of the plot a maximal ChT:DNA combination ratio of about 20:1 by weight could be derived.

It is the purpose of this communication to report briefly on additional observations regarding complexes of ChT with DNA and RNA.

Stoichiometry and Solubility of the DNA-Chymotrypsin Complex. Many proteins combine with nucleic acid to form insoluble "protein-nucleates" of indefinite composition (see: Chargaff, 1955). With ChT and DNA the protein content of the precipitate at pH <5.8 had been found to increase with the total protein content of the mixture in an asymptotic manner until it contained about 94 percent protein when the components were mixed in a ChT:DNA ratio of 600:1 (Diskina and Spitkovskii, 1958). In contrast, the kinetics of inhibition in our soluble system at pH 7.5, and with lower concentrations of the reactants, indicate that in the absence of salt all the ChT is irreversibly bound until the saturation point is reached at a maximum protein:DNA combination ratio of about 20:1. This ratio is independent of the rate and se-

quence of addition of the components. It was found also that the precipitate, formed instantaneously upon addition of nucleic acid to an acid solution of the enzyme in the range of 0.01-0.1 percent, does not occur when the mixing is carried out at a pH of ≈8 and the solution is then gradually made more acid. Such a mixture is only slightly opalescent, although some sediment may be formed upon standing. With concentrations of the order of 0.001 percent no turbidity is apparent regardless of pH.

Sedimentation Rate. A 0.06 percent solution of chymotrypsin in 0.006 M Tris buffer of pH 7.5 to which 0.003 percent DNA had been added contained material that in the ultracentrifuge sedimented from 15 to 30 times as rapidly as DNA in a 0.04 percent aqueous solution. The heavy material did not occur in the presence of 0.6 M KCl.

Complexes with RNA. Previous experiments (Hofstee, 1960 b) showed that the maximal inhibition by thymus RNA (Nutr. Bioch. Corp.) and by yeast RNA (Pabst Lab.) is of the same order as that by DNA. However, with increasing amounts of inhibitor the maximum is reached less abruptly, especially in the case of yeast RNA.

Results similar to those with thymus-RNA have now been obtained with RNA prepared by Dr. H. Fraenkel-Conrat from tobacco mosaic virus (TMV). The lower part of the inhibition curve, where the enzyme is in excess, is linear and coincides with the curve of DNA. The break in the curve is less sharp and the upper (horizontal) part indicates that the maximal inhibition is about 10 percent higher than found with DNA. However, the point of intersection of the extrapolated linear portions of the curve indicates that the combination ratio of RNA with ChT is of the same order as that of DNA.

It has been postulated (see: Fraenkel-Conrat, 1957; Schramm, 1957) that the absence of protective protein is the only reason that free TMV-RNA is much less infective than the intact virus. In view of the above results and the realization that the gross structure of the RNA-ChT complex may not be unlike that of the virus (see below), it can be expected that protection will be pro-

vided by ChT. Nevertheless, experiments by Fraenkel-Conrat (personal communication) indicate that combination with ChT has an inhibitory instead of an activating effect on the infectivity of TMV-RNA. The inhibitory action is reversible (e.g., by salt). Similar results had been obtained with spermidine (Pacific Slope Biochemical Conference, 1959) and with the complex of RNA and RNA-ase in the absence of salt (Fraenkel-Conrat and Singer, 1960).

Enzymic Activity of the Complexes. The fact that the maximal inhibition is less than 100 percent indicates that the complexes are enzymically active and suggests that the active center of the enzyme is not directly involved in the binding. Thus the complex might be considered as an enzymic unit with kinetic properties entirely different from the free enzyme.

Molecular Shape and Size. As has been pointed out previously (Hofstee, 1960 b), the combination ratio of 20:1 in the ChT-DNA complex would require the accommodation on the DNA chain of one ChT molecule with a M.W. of about 22,000 (Schwert, 1951) for each three to four nucleotide units. Thus, in view of the irreversible nature of the combination it was speculated that such a complex might bear some external resemblance to a simple linear virus like TMV of which the ratio of the protein to the nucleic acid content, and also the M.W. of the protein unit, are of the same order as in DNA-ChT. The resemblance would be even more striking with the complex of ChT and TMV-RNA. (For reviews on TMV structure see: Schramm, 1958; Fraenkel-Conrat, 1959).

Support for this speculation was found in preliminary electron micrograms made in Dr. R. C. Williams' laboratory at the University of California. The results indicated that at least part of the DNA-ChT complex in a 0.001 percent solution occurs as flexible strands of varying length up to the order of 1μ. With the above preparation of TMV-RNA the chymotrypsin complex showed particles that appeared to be shorter and less flexible than in the case of DNA. In both cases the strands are distinctly thicker and show less tendency to associate (and thus are better defined) than the strands of the free nucleic acids (see: Williams, 1957). Thus it appears that a combination of nucleic acids with ChT would aid in better visualization of the former and might enhance

the possibility of obtaining more accurate length distributions of nucleic acid preparations. The use of this proteolytic enzyme would have the additional advantage of dissociating possible aggregates of the nucleic acid strands, held together by contaminating protein (Hermans, 1959).

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