Soluble stoichiometric complexes of DNA with chymotrypsin and trypsin

Chymotrypsin

The hydrolytic activity of crystalline chymotrypsin in 10 ml 0.01 *M* methylhippurate was measured with a pH-stat at pH 7.5 and 30° in the absence of buffer. As shown in Fig. 1, addition of DNA (a "highly polymerized" preparation from calf thymus, Worthington) to the enzyme solution resulted in an inhibition proportional to the DNA concentration and inversely proportional to the ChT concentration up to the maximum of about 80% inhibition. Such a relationship indicates irreversible binding and suggests the formation of a definite (stoichiometric) complex. Although with higher ChT concentrations an insoluble complex is formed at pH < 7, in the present dilute systems no precipitate occurred in the pH range of 7.5–2.5. Furthermore, the maximal inhibition is independent of the rate of addition of DNA. These observations would distinguish such a complex from an insoluble "protein-nucleate" of indefinite composition[1]. The "saturation" point, manifested by the sharp breaks in

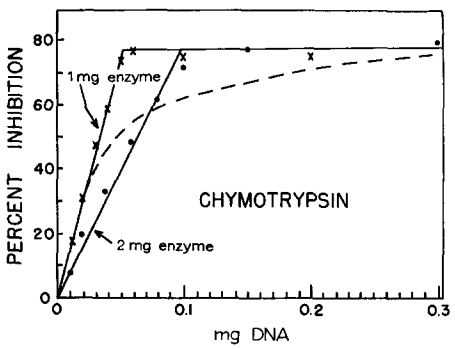

Fig. 1. Inhibition of the hydrolysis of methylhippurate (0.01 *M*) by ChT in concentrations of 1 and 2 mg per 10 ml in the presence of varying amounts of DNA at pH 7.5. The dotted line represents a dissociation curve such as that given by CMC.

the curves of Fig. 1, indicates a protein : DNA weight ratio of about 20 : 1, corresponding to approximately 4 nucleotide units per ChT molecule. In contrast to DNA, CMC (sodium salt, Hercules) which is also a potent inhibitor, combines in a dissociable ("Michaelis") manner shown by the dotted curve in Fig. 1. Thymus RNA (sodium ribonucleate, Nutritional Biochemicals Inc.) produced results intermediate between the curves of DNA and CMC. Yeast RNA (Pabst Laboratories) gave a curve resembling that of CMC. It must be noted, however, that the properties of the nucleic acids depend on the procedure of preparation[2,3]; *i.e.*, the possibility cannot be excluded that in the native state RNA combines in a manner similar to DNA.

Heating neutral solutions of DNA or RNA at 95–100° had little or no influence on the curves, but heating at alkaline pH decreased the inhibition, especially in the case of RNA. Pretreatment of yeast RNA with ribonuclease greatly decreased its inhibitory power. Addition of RNA-ase to the RNA-inhibited ChT was less effective, indicating that combination with ChT protects RNA against attack by RNA-ase. In view of the results of heat treatment and in view of the contention that RNA does

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ChT, chymotrypsin; CMC, carboxymethylcellulose.

not have the typical two-stranded structure of DNA[2-4], it is unlikely that irreversible binding is connected exclusively with such a structure. The evidence rather indicates that it is merely connected with a high degree of polymerization and regularity of structure. These properties seem to be exhibited especially by the better preparations of DNA, in contrast to the available RNA preparations[2,3].

The inhibition by the polymers is prevented by KCl in concentrations of the order of 0.1 *M*. Preliminary results with lower salt concentrations indicate that, at least in the case of DNA, this could partly be caused by an increase of the enzymic activity of the complex instead of a dissociation into free enzyme and nucleic acid.

Trypsin

The activity of 30 μg of crystalline trypsin acting in 10 ml 0.01 *M* benzoyl arginine ethyl ester–HCl was measured at 30°. At pH 6 the inhibition by DNA, showing a maximum of 74%, is much more pronounced than at pH 7.5. The inhibition curve at pH 6 displayed a sharp break similar to the case of ChT, and indicated a combination ratio of 7:1 by weight, provided the enzyme preparation did not contain inactive protein.

Some differences in nucleic acid–protein complexes extracted from tissues and those produced in the test tube can be expected, but no compelling evidence has been advanced that these combinations differ fundamentally (see ref. 4). In this connection it should be mentioned that a soluble complex, similar to a natural nucleoprotamine, is obtained when DNA and protamine are mixed in very dilute solutions[5]. It should also be noted that the protein component of tobacco mosaic virus recombines spontaneously with either the original nucleic acid to form the active virus[6] or with certain other nucleic acids of the RNA type to form inactive particles with a structure similar to that of the virus[7]. The protein:nucleic acid weight ratio of the chymotrypsin and trypsin complexes is of the same order as that of many RNA and DNA viruses[4]. Also, the accommodation of ChT on the DNA chain in a ratio of one protein molecule per four nucleotide units that together are of the order of one twentieth its size, might require considerable molecular orientation. If the structure of such complexes were similar to that of biologically active nucleoproteins, the latter could be investigated on an exact kinetic basis with the present systems serving as models. However, further studies are needed to determine whether this is the case.

This investigation was supported by grant Nr. C-2289 (C-6) from the National Cancer Institute, U.S. Public Health Service.

Palo Alto Medical Research Foundation, Palo Alto, Calif. (U.S.A.) B. H. J. HOFSTEE

[1] E. CHARGAFF, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press Inc., 1955.
[2] Symposium on Biocolloids, *J. Cellular Comp. Physiol.*, 49, Suppl. 1 (1957).
[3] *Cellular Biology of Nucleic acids and Viruses*, (T. M. RIVERS, consulting ed.) Vol. V, Special publication of New York Acad. Sci., 1957, p. 1–414.
[4] *Nucleoproteins*, 11th Chemistry Conference, Solvay Institute, Interscience Publ., 1959.
[5] P. ALEXANDER, *Biochim. Biophys. Acta*, 10 (1953) 595.
[6] H. FRAENKEL-CONRAT AND R. C. WILLIAMS, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 690.
[7] R. G. HART AND J. D. SMITH, *Nature*, 178 (1956) 1739.

Received July 18th, 1960