THE INTERACTION OF RIBONUCLEASE WITH CYTIDINE NUCLEOTIDES

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We wish to report some spectrophotometric studies on the interaction of bovine pancreatic ribonuclease A (RNAse) and an enzymically inactive derivative of RNAse with cytidine nucleotides and Zn(II) ions.

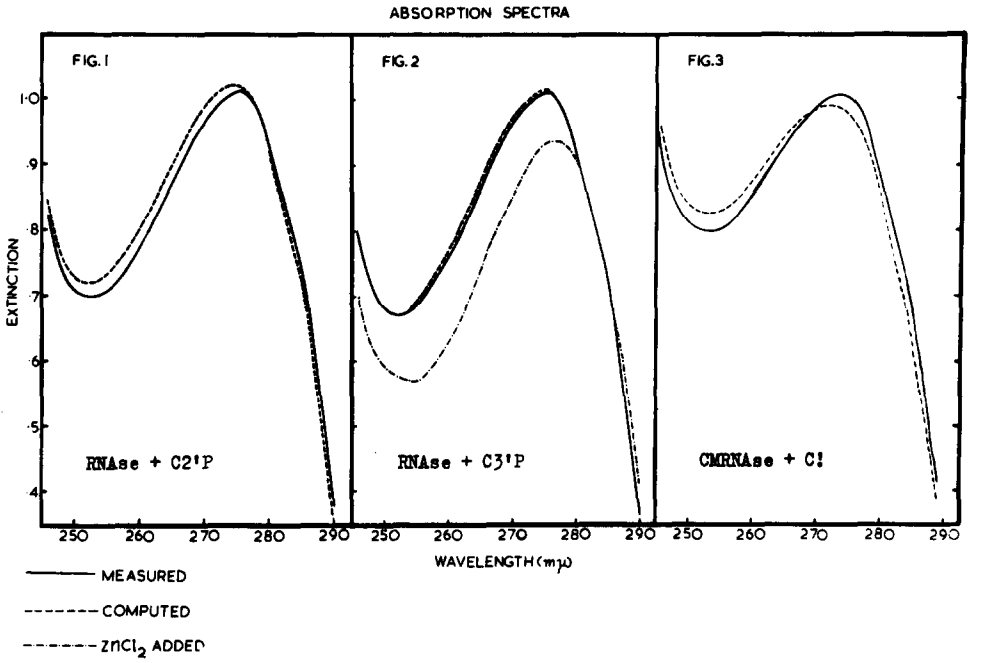
MATERIALS AND METHODS

RNAse was prepared by the method of Hirs, Moore and Stein (1953) from crystalline bovine pancreatic ribonuclease (Armour batch No. 381-062). The inactive derivative (CMRNAse) was obtained by the method of Barnard and Stein (1959) by reaction at pH 5.5 with bromoacetic acid. CMRNAse is enzymically inactive and modified solely by the carboxymethylation of one histidine residue (Barnard, personal communication).

Cytidine 2'- and 3'-phosphates, C2'P and C3'P, were prepared by the method of Harris et al (1953). Cytidine 2':3'-Phosphate (C!) was prepared by the method of Crook et al (1960). Cytidine 5'-Phosphate (C5'P) was obtained from Schwartz.

Zinc chloride was prepared as 0.1 M stock solution by dissolving ignited zinc oxide in the stoicheometric amount of HCl. Except where otherwise specified, the buffer used in all spectrophotometric measurements was 0.1 M N-ethyl morpholine/HCl, pH 6.9, I=0.2.

Absorption spectra were measured with a Hilger Uvispec model H.700 at a constant slit width of 0.6 mm. in stoppered 1 cm. quartz cells at 25°C in a thermostatted cell compartment. Corrections for dilution were made where necessary. In all experiments the concentrations of RNAse and nucleotides were $5.44.10^{-5}$ M.



RESULTS

The absorption spectra were measured for the protein and for each of the nucleotides, C2'P, C3'P, C5'P and C!, in a total volume of 2.5 ml. To each was added zinc chloride solution in increments of 0.01 ml 2.10^{-2}M (Final Zn(II) concentration approximately equimolar with protein and nucleotides). No effect was observed on the spectra of the nucleotides (Walaas, 1958), but for RNAse the first increment of zinc chloride produced a slight lowering of absorbancy in the range 240-300 mμ and further additions caused an increase in absorbancy due to precipitation of the protein.

Absorption spectra of mixtures of protein and nucleotides are shown in figures 1 and 2 where they are compared with spectra computed by assuming additivity of the absorbancies of the individual components. For C2'P, and to a lesser extent C3'P, the absorbancies in the range 250-280 mμ were less than the computed values and maxima of the measured spectra were shifted to longer wavelengths, in agreement with a report of Hummel et al (1960). The measured spectrum of the protein and C5'P showed an increase

in absorption over the entire range. The addition of zinc chloride solution to mixtures of protein and C2'P or C5'P produced the same effect observed with the protein alone. However, the addition of zinc chloride to a mixture of protein and C3'P produced a marked depression in the spectrum up to 280 mμ, an increase in absorption above 285 mμ, and a shift to longer wavelengths of the maximum and minimum. The effect increased with each addition until a five-fold molar excess of zinc chloride had been added (figure 2). Further addition of zinc chloride caused an increase in absorbancy due to precipitation. In acetate buffer pH 4.5 no alteration of the spectra of protein, C3'P, nor of a mixture of the two was caused by the addition of zinc chloride.

Interaction of the alkylated protein (CMRNAse) with C2'P, C3'P, and C5'P was substantially the same as RNAse. Addition of zinc chloride to mixtures of CMRNAse and nucleotides caused increased absorption throughout the range 240-300 mμ. Thus alkylation of the protein abolishes the effect of zinc chloride on the spectrum of RNAse plus C3'P. The spectrum of a mixture of CMRNAse and C!, a substrate of RNAse, was measured and found to differ markedly from the computed spectrum (figure 3). The first addition of zinc chloride caused a small fall in absorption; further additions caused an increase in absorption with precipitation.

DISCUSSION

The shifts observed in the absorption spectra of the mixtures of RNAse with various nucleotides are evidence that binding between the two components occurs. C3'P, which is the product of the hydrolysis of C! by RNAse, appears to be unique among cytidine nucleotides in that there is evidence, supported by kinetic experiments to be described elsewhere, for the formation of a ternary complex with Zn(II) and RNAse. Substitution of a hydrogen by a carboxymethyl group on a single histidine residue prevents the formation of this complex although the interactions of the protein with C3'P alone and the other nucleotides is unaffected. Possibly the inhibition of RNAse by zinc ions is due to the interaction of the metal with this imida-

zole. The site which binds the nucleotides is not much altered in the alkylated derivative since the interaction of CMRNAse with nucleotides is very similar to that of native RNAse. Unfortunately it is not possible to investigate the spectrum of the enzyme in combination with the substrate. However, investigation of the interaction of the inactive CMRNAse with C! should surely shed light on the combination of native RNAse with its substrate; this is being further explored in this laboratory.

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