Optical changes accompanying the enzymic breakdown of oligonucleotides

KUNITZ has found that the degradation of RNA by pancreas RNase is accompanied by spectroscopic changes which are different according to the wavelength considered: hyperchromicity in the region of 260 mμ and hypochromicity around 300 mμ. This latter decrease in extinction served as the basis for his spectrophotometric assay of pancreas RNase[1].

It is still a current belief that both effects are due to an alteration on a macromolecular level of the RNA under the influence of enzymic or chemical hydrolysing agents. From protein analogies the term *denaturation* has been used for this process[2-5]. Although an initial denaturation stage in the breakdown of RNA is not excluded, experiments with the purified RNase of *Phaseolus radiatus*[6] have shown that the decrease in A_{300} cannot be attributed solely to changes in the macromolecular structure of RNA.

When the degradation of RNA under the influence of the two RNases separately was followed under the same conditions of RNA and buffer concentration, pH and temperature, it was found that the final decrease in A_{300} was significantly greater (more than 100 % at pH 4.5) in the case of the enzyme from *P. radiatus*. Furthermore, the decrease in A_{300} immediately recommenced when the latter enzyme was added to RNA solutions which had been degraded previously by pancreas RNase until the decrease in A_{300} had ceased.

We are aware of the fact that a stronger inhibition of the pancreas enzyme by its degradation products could account partly for the smaller decrease in A_{300} found, and a closer investigation of this aspect showed indeed that the inhibition effect is not negligible. However, it does not seem sufficient to account for the large differences found between the two enzymes.

As the specificity of the RNase of *P. radiatus* is not restricted to the hydrolysis of pyrimidine nucleotide 3'-phosphate linkages only, an explanation of the extra decrease in A_{300} resulting from the action of this enzyme can be found in a further splitting of the degradation products left after the action of the pancreas enzyme. This degradation would occur most likely at the site of purine nucleotide 3'-phosphate bonds.

Investigation of the action of *P. radiatus* RNase on both dialysable and undialysable substances (the so-called *core*) formed from RNA by pancreas RNase has shown that indeed a further degradation occurs, accompanied in both cases by a decrease of A_{300}.

According to MARKHAM AND SMITH[7], the core of yeast RNA is composed of oligonucleotides with a mean chain length of 3.9 nucleotide units and it is almost completely dialysable against 2 *M* NaCl. In order to be certain that the hypochromic effect is really due to the action of the enzyme on small, dialysable oligonucleotides, the core was dialysed against 2 *M* NaCl, the dialysed substances isolated by lyophilisation, freed from NaCl by dialysis and lyophilised again. This core dialysate, in a concentration of 0.5 ‰, also showed a decrease in A_{300} when broken down by *P. radiatus* RNase.

Abbreviations: RNA, ribonucleic acid; RNase, ribonuclease; A_{260} and A_{300}, absorbance at 260 mμ and 300 mμ.

The dialysis products obtained by degradation of RNA by pancreas RNase in a dialysis bag were collected, lyophilised and submitted to the action of the *P. radiatus* enzyme. In this case it was necessary to use a higher substrate concentration (0.4 %) and again a decrease in A_{300} was found.

These observations show that the decrease in A_{300} is also characteristic for the degradation of small oligonucleotides and that it is not necessarily related to a change in the macromolecular structure of RNA, preceding a hydrolytic attack of the enzyme. Furthermore the hyperchromicity in the range of 260 mμ found by KUNITZ is not a specific feature of the degradation of RNA itself. Other authors have shown that this increase at 260 mμ is also found after the degradation of small oligonucleotides deriving from either DNA or RNA[8-11].

Our results show that the degradation of both core and dialysable fragments results in a similar increase of A_{260}. The absorption curves obtained before and after the digestion of core or of the dialysable fraction obtained by degradation with pancreas RNase resemble those obtained by KUNITZ for the breakdown of RNA itself.

It can be concluded that the spectroscopic changes accompanying the breakdown of RNA are not necessarily linked to the macromolecular structure alone of a product which is nearly intact. They are certainly also related to the hydrolytic breakdown of small fragments derived from RNA itself and probably point to some kind of interaction of certain nucleotide units when assembled in oligonucleotides resulting in the establishment of a more rigid structure.

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Deuterium exchange between myoglobin and water

In a recent series of reports from the Carlsberg Laboratory, the rate of exchange of D between H_2O and certain globular proteins has been described. The proteins studied were insulin[1], ribonuclease[2], and β-lactoglobulin[3]. To this group is now added myoglobin, a typical globular protein of which a detailed three-dimensional molecular