From the data presented here, and our earlier report[10] which gave no evidence of interconversion of the two hemins *a*, we are not inclined to discard LEMBERG's cryptohemin *a*, which appears to be identical with our hemin a_1, as artifact.

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Preliminary Notes

Latent ribonuclease activity in a ribonucleoprotein

In an account of an electrophoretic investigation of a ribonucleoprotein preparation obtained from *Escherichia coli* it was reported that exposure of the nucleoprotein to high concentrations of urea effected a separation of protein and ribonucleic acid (RNA), presumably by the rupture of hydrogen bonds between the two moieties[1]. The present communication contains a description of a second event which takes place in the same system, namely, the degradation of the RNA. This degradation appears to be the result of an enzymic process initiated by the action of urea.

It was initially observed that treatment of the nucleoprotein with 4 *M* or 7–8 *M* urea caused the appearance of two electrophoretically distinct ultraviolet-absorbing components, one migrating with the velocity of free RNA, and the other more slowly[1]. The slow component showed a mobility in starch, at pH 8.4, similar to that of a mixture of ribonucleotides. Eluted from the starch block, it was found to contain organically bound phosphorus. It may be noted that polymerized nucleic acid has previously been shown to have a higher electrophoretic mobility than nucleotides at pH 8.6[2].

When the duration of the urea treatment was held to a minimum (30 min before the start of electrophoresis, in these experiments), the slow component comprised only a small part of the ultraviolet-absorbing material. With the passage of time, however, the slow component increased in quantity, while the fast component diminished and eventually disappeared, indicating a progressive degradation of RNA to smaller fragments.

The degradation was then investigated by following the appearance of acid-soluble ultraviolet-absorbing material at 0–3°. In the absence of urea, nucleoprotein solutions were stable in the cold for at least one week, and precipitation by 0.1 *M* $HClO_4$ was complete (98–99%). The addition of urea initiated a release of acid-soluble material which continued until all of the RNA was degraded.

The rate of degradation increased with the concentrations both of urea and of the nucleoprotein, and was markedly dependent on the NaCl concentration of the solution. Degradation proceeded slowly in the absence of salt, rapidly in 0.03 *M*, 0.1 *M* and 0.3 *M* NaCl, and was virtually abolished in 1.0 *M* NaCl.

This may be a general salt effect, since 0.05 *M* sodium phosphate, pH 7.5 (ionic strength 0.12), also allowed rapid degradation. Salt caused no degradation in the absence of urea. It was also observed that the degradation was inhibited by the sodium salts of polyacids (polyglutamic, polyacrylic and deoxyribonucleic acids) in the presence of urea but in the absence of salt. This inhibition was reversed by 0.3 *M* NaCl.

To test for the presence of a nucleolytic enzyme, RNA (commercially obtained, deproteinized and dialyzed) was added to the nucleoprotein. This RNA was not degraded in the absence of urea, whether or not NaCl was present. When urea was added, both the nucleoprotein and the RNA were degraded slowly in the absence of salt, and rapidly in 0.3 *M* NaCl. Deoxyribonucleic acid was not hydrolyzed under the same conditions. Thus the nucleoprotein appears to contain a latent enzyme which is liberated by urea treatment and shows a certain degradative specificity towards RNA.

The following experiment showed the activity to be a property of the protein moiety. The nucleoprotein was allowed to undergo self-digestion in urea and was dialyzed against water, effecting the complete removal of ultraviolet-absorbing material. The residue was then tested and found to be capable of degrading RNA both in the presence and absence of urea.

The identity of the enzyme has not yet been established but, as shown below, it bears a considerable resemblance to pancreatic ribonuclease. Its inability to digest deoxyribonucleic acid indicates that it is not an unspecific phosphodiesterase. Polynucleotide phosphorylase[3,4] is also ruled out, by the following observations: (a) RNA is degraded in the absence of inorganic phosphate. (b) There is no exchange of radioactive orthophosphate with adenosine diphosphate under conditions where degradation of RNA occurs (ref. [4], assay C*).

On the other hand, the enzyme of the nucleoprotein shows at least a qualitative resemblance to pancreatic ribonuclease in several respects. Among them are the effects of salt[5,6] and polyacids[7] described above and the fact that activity is maintained in high concentrations of urea[8]. An additional similarity was found in the heat stability[9]. As is true of pancreatic ribonuclease, the nucleoprotein retained a significant degree of nucleolytic activity after being heated for 15 min at 100° at pH 2.9; the same treatment at pH 8.5 completely destroyed the activity. In view of these similarities, it appears justified to refer tentatively to the enzymic activity of the nucleoprotein as ribonuclease activity. The observed effects of salt and polyacids can be reconciled with a scheme in which electrostatic forces participate in the formation of enzyme-substrate and enzyme-inhibitor complexes.

As to the nature of the initiation of enzyme activity by urea treatment, it is plausible to suppose that this is brought about by the dissociation of hydrogen bonds between the enzyme and RNA. According to this point of view, the enzyme, in its latent form, would constitute part of a ribonucleoprotein. It would become enzymically active only after being separated from the RNA.

It is not yet possible to assess the significance of the presence of a latent ribonuclease in this ribonucleoprotein. Perhaps this enzyme plays a special role in nucleoprotein metabolism or protein synthesis. It might conceivably function in stripping RNA away from completed polypeptide chains, being activated in nature in a manner as yet unknown.

Alternatively, this ribonuclease might not occupy a special position, but simply be one of a large number of proteins at one of the terminal stages of synthesis. If so, the nucleoproteins should contain other enzymes as well; although these enzymes, unlike ribonuclease, might be inactive in high concentrations of urea. In this connection, a few exploratory experiments have been made to test for the appearance of several other enzymes. The results have been negative, but the experiments have not been definitive and are being continued.

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