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

model has recently been proposed by KENDREW *et al.*[4], based on X-ray analysis by the method of isomorphous replacement.

A sample of crystalline sperm-whale metmyoglobin was generously presented by Dr. J. C. KENDREW. Analysis by Dr. A. B. EDMUNDSON[5] defined the amino acid composition and he has kindly permitted us to quote it as follows: Asp_8, Glu_{19}, Gly_{11}, Ala_{17}, Val_8, Leu_{18}, $Ileu_9$, Ser_6, Thr_5, Met_2, Pro_4, Phe_6, Tyr_3, His_{12}, Lys_{19}, Arg_4, Try_2 $(CONH_2)_7$. The mol. wt., based on the empirical formula, is 17,830 and the 153 amino acid residues are arranged in a single peptide chain. In the isoelectric protein, each molecule contains by calculation 268 exchangeable H atoms (atoms that are O- or N-bound), 148 of which are contributed by the –CO–NH– groups of the peptide backbone.

Details of the method of D exchange have been described[6,7]. In preliminary experiments, we found that replacement of exchangeable H atoms by D was incomplete when myoglobin was dissolved in 99.73 % D_2O in the isoelectric region of pH (pH 7) at 38°. Fig. 1 illustrates that at pH 7, the rate of D uptake rose to reach a plateau at which n, the number of H atoms exchanging per mole of protein, had a value of approximately 244 as compared to the calculated 268. It seems, thus, that under these conditions 24 atoms do not exchange at a finite rate. We suggest, on the basis of considerations discussed previously[8], that these non-exchanging atoms represent imide H atoms of a backbone segment or segments, comprising 16 % of the peptide chain, rigidly stabilized in a H-bonded ordered structure.

Since, on the other hand, replacement of all exchangeable H atoms by D is accomplished at pH 8.8 on incubation in D_2O at 38° for 10 h or more (Fig. 1), the protein so treated and subsequently completely dried may be used to study back-exchange of D with H by dissolving it in water. Fig. 1 illustrates the results of 2 such experiments which indicate that at pH 8.8 D is exchanged with H at a rate and to an extent comparable to its initial uptake at this pH and temperature (38°). At pH 7 the rate of back-exchange resembles that of uptake at pH 7 and only approximately 248 of the 264 D atoms taken up per mole of protein are exchanged when a steady state is reached. Apparently, the structural change of the molecule which permits 264 groups to exchange at pH 8.8, as compared to 244 at pH 7, is almost completely reversible and 16 D atoms are "buried" within the molecule when the pH is changed from pH 8.8 to pH 7.

To substantiate this an attempt was made to recover these "buried" D atoms. After uptake of D at pH 8.8 for 20 h, and back-exchange with H (of H_2O) at pH 7 for an additional 22 h, dry myoglobin was dissolved in water buffered at pH 8.8 (all of the exchanges were conducted at 38°). Fig. 2 illustrates the rate at which the "buried" D atoms were exchanged for H at pH 8.8 and indicates that approximately 13 were recovered under these circumstances.

Finally, we attempted to obtain an estimate of the percent helically ordered structure of the peptide backbone of myoglobin at 0°. We based this estimate on the following assumptions: that all side-chain replaceable H atoms and imide H atoms of unordered segments of the backbone exchange instantaneously and that the relatively slowly exchanging H atoms represent the imide H atoms of H-bonded, helically ordered segments of the peptide backbone[8]. Myoglobin, of which all the exchangeable H atoms had been replaced by D by incubation in D_2O at 38°, pH 8.8, was used. It was dissolved in water buffered at pH 7 and pH 8.8 and incubated at 0°.

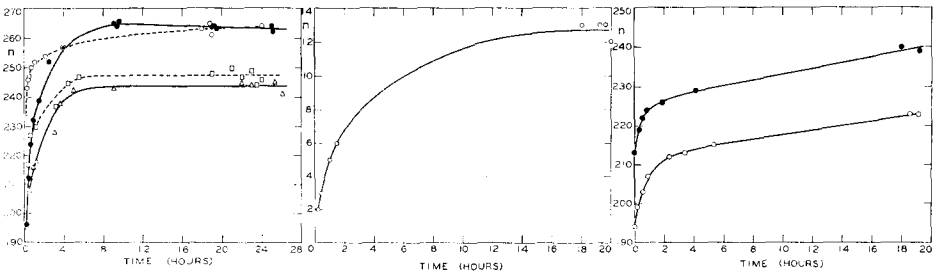

Fig. 1. Fig. 2. Fig. 3.

Fig. 1. Rate of deuterium uptake and back-exchange at pH 7 and 8.8. ●, D uptake at pH 8.8; ○, D back-exchange at pH 8.8; △, D uptake at pH 7; □, D back-exchange at pH 7 after uptake at pH 8.8 for 20 h. All experiments conducted at 38°.

Fig. 2. Rate of exchange of "buried" D atoms at pH 8.8, 38°.

Fig. 3. Rate of exchange of D at o° ●, pH 8.8 ○, pH 7.

Fig. 3 shows that under these circumstances, at pH 7, 193 atoms are exchanged instantaneously leaving 75 slowly exchanging atoms. Thus the imide H atoms of 75 of 148 amino acid residues which have imide H atoms may be considered, under the experimental conditions, to participate in a H-bonded structure, and if this is assumed to be helically ordered, approximately 51 % of the backbone is so ordered. At pH 8.8 only 56 atoms exchange slowly and the helically ordered portion of the backbone is estimated to be 38 %. These estimates should be regarded as minimum ones (pH, 0°, etc.) valid for molecules in aqueous solution under the given conditions. An estimate of 70 % α helix has been made by KENDREW *et al.*[5] based on X-ray analysis of crystalline myoglobin. A strict comparison with our values is hardly possible due to the difference in the state of the molecules. The rough agreement obtained at pH 7 is, however, interesting. Further details of this study will be reported and discussed more extensively elsewhere. Reference is made to 3 papers on the D exchange of poly-DL-alanine[9,10,11] in which the relation between pH or T and percentage H-bonded structure in peptides has been analyzed.

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