TREATMENTS AFFECTING THE ULTRAVIOLET ABSORPTION SPECTRUM OF RIBONUCLEIC ACID FROM THREE SOURCES

by

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Nucleic acid absorbs ultraviolet light strongly with a maximum at about 260 mμ. Many attempts have been made to use measurements of the intensity of absorption in this region as a means of determining the amount of nucleic acid in tissues or extracts, but they have not been wholly successful because other widely distributed substances, *e.g.* ascorbic acid[1] also absorb here and satisfactory evidence that they have been removed is seldom given. Furthermore, the intensity of the absorption depends on the state of aggregation of the nucleic acid so that if a treatment alters the aggregation there is a change in absorption, although the total amount of nucleic acid and its split products has not been altered. These statements apply to both ribose and desoxyribose nucleic acid; in the remainder of this paper only ribonucleic acid is referred to.

Absorption curves published by KUNITZ[2] show a very slight increase at 260 mμ when yeast nucleic acid (YNA) is digested with pancreatic ribonuclease (PRNase). With nucleic acid made from tobacco mosaic virus (TMV), OSTER AND GRIMSSON[3] found an 8% increase after PRNase action and a 13% increase after exposure to alkali. An increase after PRNase action on YNA has also been found by MALLETTE AND LAMANNA[4] and after exposure to 1 *M* $HClO_4$ by OGUR AND ROSEN[5].

TSUBOI[6] observed a 20% increase in the absorption by YNA at 260 mμ after PRNase action or heating for 20 min at 90° in 50 g/l trichloracetic acid and a 32% increase after hydrolysis for 15 h at 37° by 0.5 *N* NaOH. The results on four samples of commercial YNA and two samples of nucleic acid from mouse liver were similar. YNA and liver nucleic acid, and a series of products made from them by partial hydrolysis, were examined by MAGASANIK AND CHARGAFF[7]. They concluded that the increase in absorption was a feature of the breakdown of products of large molecular weight and particularly of those containing guanine. They expressed doubt about the relevance of the increase found by others after PRNase action because they regarded a 20% increase as the minimum that should be considered real.

We have investigated the effects of different extents of hydrolysis by leaf ribonuclease (LRNase) and other agents on the absorption spectrum of nucleic acids from yeast, tobacco mosaic virus (TMV) and nucleoprotein from uninfected tobacco leaves (NP). All behave similarly and the increase in absorption on partial hydrolysis, by any of the agents used, parallels the extent of hydrolysis.

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EXPERIMENTAL

Materials

Nucleic acids were made by the methods described by HOLDEN AND PIRIE[8], TMV by the method of BAWDEN AND PIRIE[9] and NP by that of PIRIE[10]. LRNase was made from pea seedlings by the method of HOLDEN AND PIRIE[11]; PRNase was a commercial product (Armour & Co. Ltd.).

Methods

The absorption spectra were measured at about 20° in 1 cm quartz cells on a Unicam (Cambridge) spectrophotometer.

The extent of hydrolysis was determined by precipitating samples of the nucleic acid solutions, at the time at which they were diluted for spectrophotometry, with uranyl nitrate and trichloracetic acid and determining the P content of the fluid and precipitate[12].

RESULTS

In Table I the intensity of absorption at 258 mμ of nucleic acids made from the three sources is set out, and also the increases brought about by different treatments.

TABLE I

EFFECT OF DIFFERENT TREATMENTS ON THE INTENSITY OF ABSORPTION AT 258 mμ OF NUCLEIC ACIDS FROM FIVE SOURCES

The treatments were carried out in the manner described in the caption to Fig. 1. E_p^{20} is absorption ($\log_{10} I_0/I$) of a 1 cm layer of solution molar with respect to P.

Preparation	*Treatment*	E_p	*% hydrolysis i.e. % of total P which is UrTCA soluble*
YNA	—	7,700	0
	PRNase, 37°, 18.0 h	9,800	32
	LRNase, 37°, 18.0 h	11,100	89
	N-NaOH, 37°, 18.0 h	10,900	100
	N-$HClO_4$ 18°, 0.5 h	8,900	8
	N-$HClO_4$ 18°, 1.5 h	9,700	31
	N-$HClO_4$ 18°, 18.0 h	10,600	100
purified commercial YNA	—	8,900	0
	PRNase, 37°, 18 h	9,800	32
	LRNase, 37°, 18 h	9,300	21
	LRNase, 37°, 18 h	9,800	36
	LRNase, 37°, 18 h	10,200	90
TMV nucleic acid	—	8,300	0
	PRNase, 37°, 18 h	10,050	42
	LRNase, 37°, 18 h	12,200	100
	N-NaOH, 37°, 18 h	10,300	70
	N-$HClO_4$ 18°, 18 h	11,200	100
Tobacco leaf nucleic acid	—	8,600	0
	PRNase, 37°, 18 h	10,600	56
	LRNase, 37°, 18 h	11,200	100
	N-NaOH, 37°, 18 h	11,800	100
	N-$HClO_4$ 18°, 18 h	11,600	100
YNA "core"	—	10,000	< 5
	LRNase, 37°, 18 h	12,600	100
	N-NaOH, 37°, 18 h	12,100	85
	N-$HClO_4$ 18°, 18 h	13,200	100

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From this it is clear that our results agree with those already published and summarised above. It is also clear that the smaller increase that PRNase causes compared to the increase caused by NaOH or $HClO_4$ is in agreement with the smaller apparent percentage hydrolysis using precipitability by uranylnitrate and trichloracetic acid as an index of this. Hydrolysis by LRNase, when allowed to go to completion causes as large an increase as hydrolysis by the other agents, but if the action is stopped at an intermediate point, where hydrolysis similar to the limit with PRNase has been reached, the increase is similar to that with PRNase. Table I also shows that the acid precipitable residue, or "core" that remains after PRNase action gives the normal increase when digested by LRNase. We have chosen this result for fuller presentation in Fig. 1 because few absorption curves of "core" have been published.

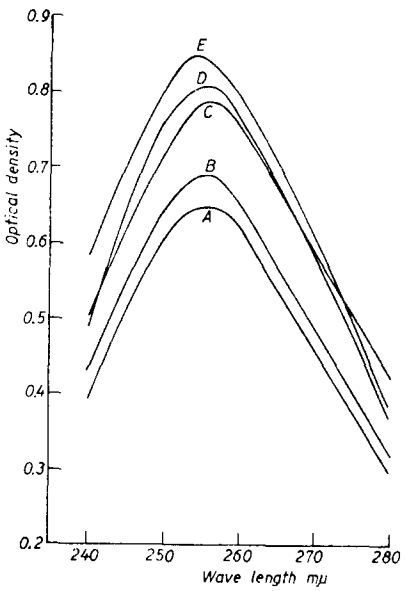

Fig. 1. The effect of LRNase, acid and alkali on the ultraviolet absorption curve of YNA "core". Curve A: A soln. of YNA "core" at 2 mg P/l in 2.5 m*M* sodium citrate (pH 6). B: A soln. of "core" at 20 mg P/l in 25 m*M* citrate incubated for 18 h at 37° and diluted in 1 in 10 with water. C: As (B), but with LRNase 0.1 U/ml[11] present, during incubation. D: A soln. of "core" 20 mg P/l incubated for 18 h at 18° in *N* $HClO_4$. Neutralized with 2 *N* NaOH and 25 m*M* sodium citrate (pH 6) added to a conc. of 12.5 m*M* and then diluted 1 in 5 with water. E: A soln. of "core" 20 mg P/l incubated for 18 h at 18° in *N* NaOH. Neutralized with 2 *N* $HClO_4$ and 25 m*M* sodium citrate (pH 6) added to a conc. of 12.5 m*M* and then diluted 1 in 5 with water.

No detailed proposals have been made for the reasons for this increase in absorption but both MAGASANIK AND CHARGAFF[7] and MARKHAM AND SMITH[13] agree that it is the consequence of a relaxation of cross links in large molecular aggregates which had restricted the resonance of which nucleotide rings are capable when separated. The effect of other agents which might be expected to relax these links in nucleic acid without producing acid soluble material are, therefore, of interest. Choice is limited by the necessity for using agents with little or no absorption about 260 mμ because the treatments needed to remove the agent might well have more effect than the agent itself. This rules out agents such as pyridine[14], phenol[15] and strontium nitrate[16] all of which affect many types of large molecule and are known[14,15,16] to dissociate some nucleoproteins, but urea, which also dissociates nucleoproteins[17], absorbs sufficiently little at 260 mμ to permit the study of its effect on nucleic acid without the necessity for removing the reagent. The results have been unsystematic. A few YNA preparations have shown an increase of from 4 to 15% after exposure for a day to 7 *M* urea at pH 7 but with many others there has been no effect. Preparations of nucleic acid from TMV have not been affected. With no preparation of YNA has the intensity of absorption been diminished by the treatment.

This effect, or lack of effect, of urea on YNA and TMV nucleic acid stands in interesting contrast to its effect on intact TMV and potato virus X for, during the action on these nucleoproteins, BAWDEN AND PIRIE[17] observed a diminution in the absorption at 260 mμ. Fig. 2 shows this phenomenon with TMV and it also shows that the dimi-

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nution is substantially complete after 25 min. After that time no YNA preparation had shown any increase in absorption. It seems probable therefore, that the effect is due either to a change in the protein moiety or to breakage of the protein : nucleic acid link. The latter origin is perhaps the more probable because BAWDEN AND PIRIE[17] found no diminution during the action of urea on tomato bushy stunt virus and with it urea does not separate protein from nucleic acid.

In an attempt to get further evidence for the effect of separation we have studied the absorption spectra of NP after various types of incubation which[10] separate protein from nucleic acid, but the results are confused by the precipitation of denaturated protein which carries with it, in the first stages, some of the nucleic acid. They lead therefore to no definite conclusion. During the action of urea on NP however, there is no precipitation and also there is no significant change in the intensity of absorption.

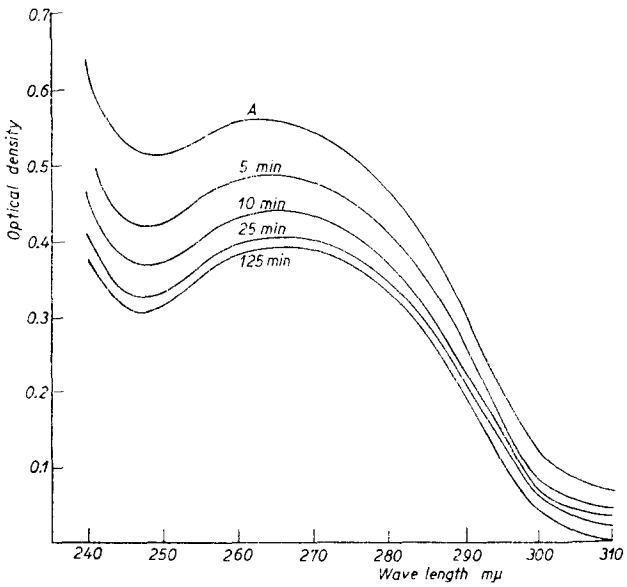

Fig. 2. The effect on the absorption spectrum of TMV of different durations of contact with 6.6 *M* urea. The absorption curves of two solutions are superposed in curve A. Each contained 195 mg TMV (that is 1.0 mg P) per litre and 1 m*M* pH 7.5 phosphate buffer and one of them contained 0.66 *M* urea. The other curves contained these final concentrations of TMV etc., but they were made by the tenfold dilution of mixtures containing TMV, phosphate and urea that had been kept at 0° for the time specified.

The idea that the anomaly with TMV lies in the nature of the union between protein and nucleic acid gains some support from the magnitude of the absorption at 260 mμ. Thus the solution used in the experiment summarised in Fig. 2 contained 1 mg P/l and gave log $I_0/I = 0.56$. This is a value that is only reached by most YNA and NP solutions when they contain 2 mg P/l. This type of evidence cannot, however, be clearly interpreted until more is known about the absorption with proteins free from nucleic acid and about the importance of scattering as a cause of opacity at 260 mμ[15].

There seems to be general agreement that proteins absorb more strongly at 260 mμ than would be expected from the sum of the absorptions of their constituent amino acids, though the extent to which this extra absorption is caused by inter-peptide links

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converting the peptide chains into a fabric is under dispute[18,19]. We are not aware of any investigation of the effect of urea on the absorption although it is an agent that might well break the type of link postulated. We have, therefore, compared the spectra, in the 260 mμ region, of ovalbumin and horse serum albumin before and after exposure at 4 g/l to 6.6 *M* urea in 20 m*M* pH 7 citrate for 24 h at 0° and find no change outside the experimental error. We conclude, therefore, that urea denaturation is not sufficient to remove the excess absorption with albumins and that the effect found with TMV is not found generally with proteins. A fuller investigation of the phenomenon might well shed light on the nature of the linkage between protein and nucleic acid in TMV.

SUMMARY

The intensity of absorption in the 260 mμ region by three different types of ribonucleic acid and by the fraction of YNA that resists attack by pancreatic ribonuclease, is increased during several types of hydrolysis to an extent that parallels the extent of hydrolysis.

Exposure to strong urea solutions has a small and irregular effect on the absorption by nucleic acid but diminishes the absorption by TMV.

RÉSUMÉ

L'intensité d'absorption aux environs de 260 mμ de trois types différents d'acide ribonucléique et de la fraction de l'acide nucléique de la levure qui résiste à l'attaque par la ribonucléase du pancréas, augmente au cours de plusieurs types d'hydrolyse, parallèlement au degré d'hydrolyse.

Le contact avec des solutions concentrées d'urée a une action faible et irrégulière sur l'absorption de l'acide nucléique mais diminue l'absorption du virus de la mosaïque du tabac.

ZUSAMMENFASSUNG

Die Absorptionsintensität im Bereich von 260 mμ, die durch 3 verschiedene Arten von Ribonukleinsäure und durch die YNA-Fraktion, die resistent gegen Pankreasribonuklease ist, hervorgerufen wird, wächst während verschiedener Formen der Hydrolyse in einem Ausmass, das dem Ausmass der Hydrolyse parallel geht. Die Behandlung mit starken Harnstofflösungen hat einen kleinen und unregelmässigen Effekt auf die Absorption durch die Nukleinsäuren, verringert aber die Absorption von TMV.

REFERENCES

1 H. MOHLER AND H. LOHR, *Helv. Chim. Acta*, 21 (1938) 485.
2 M. KUNITZ, *J. Biol. Chem.*, 164 (1946) 563.
3 G. OSTER AND H. GRIMSSON, *Arch. Biochem.*, 24 (1949) 119.
4 M. F. MALLETTE AND C. LAMANNA, *Arch. Biochem. Biophys.*, 51 (1954) 217.
5 M. OGUR AND G. ROSEN, *Arch. Biochem.*, 25 (1950) 262.
6 K. K. TSUBOI, *Biochim. Biophys. Acta*, 6 (1950) 202.
7 B. MAGASANIK AND E. CHARGAFF, *Biochim. Biophys. Acta*, 7 (1951) 396.
8 M. HOLDEN AND N. W. PIRIE, *Biochem. J.* (1955, in the press).
9 F. C. BAWDEN AND N. W. PIRIE, *Biochem. J.*, 37 (1943) 66.
10 N. W. PIRIE, *Biochem. J.*, 47 (1950) 614.
11 M. HOLDEN AND N. W. PIRIE, *Biochem. J.* (1955, in the press).
12 M. HOLDEN AND N. W. PIRIE, *Biochem. J.* (1955, in the press).
13 R. MARKHAM AND J. D. SMITH, in *The Proteins* ed. by H. NEURATH and K. BAILEY, Academic Press, Inc. New York, Vol. II A, (1954) p. 1.
14 F. C. BAWDEN AND N. W. PIRIE, *Proc. Roy. Soc.* B, 123 (1937) 274.
15 F. C. BAWDEN AND N. W. PIRIE, *Biochem. J.*, 34 (1940) 1278.
16 N. W. PIRIE, *Biochem. J.*, 56 (1954) 83.
17 F. C. BAWDEN AND N. W. PIRIE, *Biochem. J.*, 34 (1940) 1258.
18 G. H. BEAVEN, E. R. HOLIDAY AND E. M. JOPE, *Discussions Faraday Soc.*, 9 (1950) 406.
19 P. DOTY AND E. P. GEIDUSCHEK, in *The Proteins*, ed. by H. NEURATH and K. BAILEY, Academic Press, Inc. New York, Vol. I A, (1953) 393.
20 E. CHARGAFF AND S. ZAMENHOF, *J. Biol. Chem.*, 173 (1948) 327.

Received October 12th, 1954