THE CHANGE OF ULTRAVIOLET ABSORPTION OF THYMO-NUCLEOPROTEIN AND THYMO-NUCLEIC ACID WITH pH

by

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It has been shown earlier[1] that a thymo-nucleoprotein solution has a greater light absorption at 260 mμ at a pH above 11 than in a neutral solution.

This phenomenon has now been more closely investigated.

Thymo-nucleoprotein

Thymo-nucleoprotein was prepared[1] according to MIRSKY AND POLLISTER[2,3]. Samples taken from a solution containing nucleoprotein in 1 *M* NaCl and phosphate buffer (pH 6.3, ionic strength 0.05) were diluted with buffer solutions to different pH values keeping the concentration of NaCl at 1 *M*. Acetate, phosphate and NaOH-glycine buffers were used. The light absorption was measured in a Beckman spectrophotometer between 400 and 230 mμ against the solvent of each solution. Typical curves are shown in Fig. 1.

The maximal extinction value of each curve is plotted against the pH of the solution (Fig. 2). These maxima were obtained at a wavelength between 258 and 260 mμ.

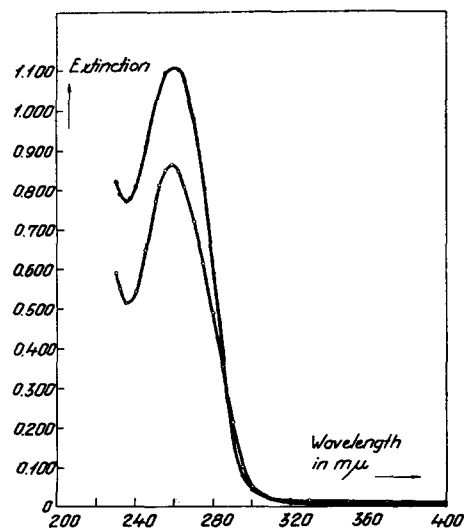

Fig. 1. Ultraviolet absorption for a preparation of thymo-nucleoprotein at pH 6.3 (unfilled circles) and pH 12 (filled circles). See also Fig. 2.

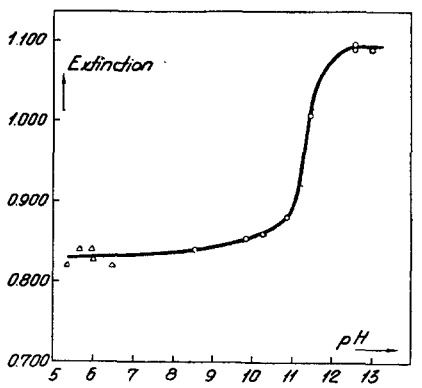

Fig. 2. The change of maximal ultraviolet absorption (wavelength = 258–260 mμ) with pH for a preparation of thymo-nucleoprotein (the same as in Fig. 1).
Triangles—phosphate buffers
Circles—NaOH-glycine buffers

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The maximal values were corrected for unspecific absorption[4]. The absorption between 400 and 370 mμ is, however, never more than 1.5% of the maximal absorption. It has also been observed that when the maximal absorption increases the unspecific absorption decreases.

The maximal extinction increases quite sharply at about pH 11.3. The uncertainty in pH was about ± 0.15, owing to the difficulty of measuring pH with a glass electrode in solutions with high NaCl content. For checking, the buffers were measured both with and without NaCl and the results compared with values calculated from tables.

The absorption at pH 6.3 was found to be 76% of that at pH 12. The quotient between extinction at pH 6.2 and 260 mμ and mgN/ml was 55, which is the value usually given (see earlier article[1]) for thymo-nucleoprotein.

When the nucleoprotein was dialyzed against 0.1 *M* NaOH only traces of highly absorbing substances went through the membrane.

Thymo-nucleic acid

The light absorption in the nucleoproteins, is mainly due to the nucleic acid. Separate measurements were therefore carried out on the acid.

Nucleic acid was prepared from fresh nucleoprotein preparations using the method of GULLAND, JORDAN AND THRELFALL[5], but working with less material.

Preparation of sodium desoxypentose nucleate

To 100 ml 0.5% freshly prepared nucleoprotein solution in 1 *M* NaCl + phosphate buffer enough salt was added to make the NaCl concentration close to 10%. The solution was then stirred with a common glass rod stirrer for some hours together with 35 ml chloroform and 10 ml amyl alcohol. Air was bubbled through the solution in order to take away the chloroform. The solution was centrifuged for about one hour at 9,500 r.p.m. in tubes containing about 15 ml. The denaturated protein afterwards lay as a film on the surface or stuck to the upper part of the tube wall.The underlying fluid was carefully sucked off with a pipette.

A fresh portion of chloroform together with some amyl alcohol was added and the whole procedure repeated.

Eight or nine stirrings with chloroform and amyl alcohol were found to be necessary to give a good product. After the two last stirrings the centrifugation was carried out at 27,000 r.p.m. for 20–30 minutes.

A volume of methyl alcohol, equal to the volume of the aqueous solution was added, giving a white precipitate of sodium nucleate which was centrifuged down at about 3,000 r.p.m. and collected.

The nucleate was washed twice with ethyl alcohol and once with ether.

The nitrogen content in the preparations of the nucleic acid was found to be 16.3%. The nucleate itself contained 15.1% N. Sodium and chlorine were determined as a check on the amount of NaCl precipitated with the nucleate. The nitrogen content is in good agreement with the values given by GULLAND. The N/P value was found to be 3.5 in agreement with the values given by both GULLAND and by CHARGAFF *et al.*[6]. CHARGAFF, however, records lower nitrogen value. N was determined by the Kjeldahl method, P according to KING, Na as sulphate determined by weight and Cl potentiometrically.

The nucleate preparation gave no reaction with ninhydrin.

A closer investigation of this method of preparation is under progress.

One of the preparations of sodium nucleate was dissolved in distilled water and each of two others in 1 *M* NaCl. The strong salt solutions were used to make a comparison possible with the nucleoprotein solutions in 1 *M* NaCl.

Samples were taken from the nucleate solutions and diluted with buffer solutions of

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different pH. For each pH the absorption between 400 and 232 mμ was determined in the Beckman spectrophotometer as described above. The unspecific absorption between 400–370 mμ was never more than 2% of the maximal value—decreasing, when the latter increased, to a value of 1% or less.

One of the curves resulting from the plot of the maximal absorption values against pH is seen in Fig. 3. The solution contains 0.0048 mg N/ml and 1 *M* NaCl.

The observed increase in absorption occurs for all the three preparations of sodium nucleate investigated between 11.0 and 11.2. For the two solutions containing 1 *M* NaCl the difficulty of measuring pH are naturally the same as those mentioned above.

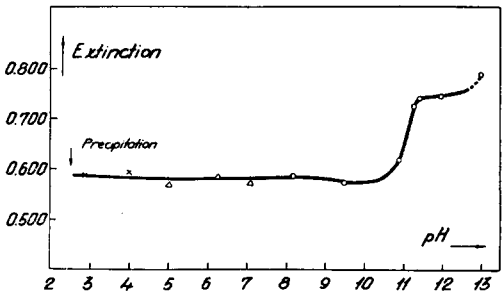

Fig. 3. The change of maximal ultraviolet absorption (wavelength 258–260 mμ) with pH for a preparation of sodium desoxypentose nucleate. See also Fig. 4.
Crosses—acetic acid buffers
Triangles—phosphate buffers
Circles—NaOH-glycine buffers

In all three cases the maximal absorption at pH 6.3 is 75% of that at pH 12. The quotient between maximal extinction and nitrogen content in mg/ml for the nucleate in Fig. 3 at these two pH values is 121 and 160 respectively. Compared with the value of this quotient, 55, for nucleoprotein, 121 would give a nucleic acid content of 43% in the nucleoprotein, in good agreement with earlier results[1]. (16.3% N in the nucleic acid).

The two other nucleic acids give quotients which are lower, but not by more than 10%. The nitrogen value has been determined in these two cases, however, on very small quantities of material.

When the pH of the solutions of sodium nucleate, which had been buffered at pH 11.3 or higher, was brought down to pH 9 or less, the absorption at 260 mμ decreased, but it was still higher than for solutions which had been kept at the lower pH values. The absorption at pH 6.3 was about 11% higher than for the original solution (see Fig. 4).

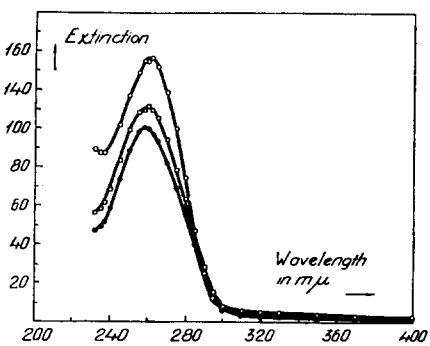

Fig. 4. Ultraviolet absorption for a preparation of sodium desoxypentose nucleate at pH 6.3 (filled circles) and pH 12.0 (unfilled circles). The third curve (squares) gives the absorption at pH 6.4 after neutralization from pH 12.0. The maximal extinction at pH 6.3 = 100.

The back neutralization was carried out by adding phosphate buffer to the solution of nucleate in NaOH-glycine buffer and subsequently adding 0.1 *M* HCl. The absorption was measured one hour and 24 hours after the neutralization, keeping the solution at room temperature. No change was observed with time. The solution was kept for different time at the high pH. Measurements, keeping the solution alkaline from 10 min up to 4 hours showed very little or no change in the reduction of absorption after neutralization. If the solutions were kept strongly alkaline for several days the reduction decreased.

The sensibility of the absorption to an increase in pH is decreased if the nucleate is dried over silica gel at 60° C for 24 hours.

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DISCUSSION

Investigations of the absorption of the purines and pyrimidines at different pH values have been carried out by HOLIDAY[7], HEYROTH AND LOOFBOUROW[8] LOOFBOUROW AND STIMSON[9], STIMSON AND REUTER[10,11], HOTCHKISS[12], and by CAVALIERI, BENDICH, TINKER AND BROWN[13]. Their values show mutual agreement*.

The absorption of the nucleic acids in the ultraviolet region is due to the purines and pyrimidines, *i.e.* in the case of thymonucleic acid—adenine, guanine, thymine and cytosine. Of these only cytosine gives an increase in absorption when a change is made from a neutral to alkaline solution. The other three compounds show a decreased absorption.

STIMSON AND REUTER[10] have measured the absorption of commercial yeast nucleic acid (EASTMAN), purified according to LEVENE, and thymus nucleic acid, prepared according to FEULGEN, in water, 0.1 *M* HCl and 0.1 *M* NaOH. They obtained extinction values which were slightly lower for the solutions in NaOH than for the water solutions. However, the absorption of the nucleic acids did not show the same response to pH as the free pyrimidines. The present author has repeated the measurements on commercial yeast nucleic acid (E. MERCK). The maximal absorption of the nucleic acid showed an increase with increasing pH. The density readings were, for instance, in neutral solution 0.758 at 258 mμ and in 0.1 *M* NaOH 0.838 at 260 mμ. The absorption at 400 mμ, however, at the same time increased from 0.007 to 0.017. At intermediate pH values the preparation showed intermediate increased absorption.

TSUBOI[14] has investigated nucleic acid from mouse liver. He gives a curve for the absorption after treating the acid with 0.5 *M* NaOH for 30 minutes at 95°. This curve shows an increase in absorption at 260 mμ of about 22% compared with that in a neutral solution.

If the values for the content of the different purines and pyrimidines given by CHARGAFF *et al.*[6], taking a mean value from their Table IV, are combined with the values for extinction given by HOTCHKISS[12] the values in Table I are derived.

TABLE I

THEORETICAL EXTINCTION OF THYMO-NUCLEIC ACID CALCULATED FROM THE EXTINCTIONS OF THE SINGLE PURINES AND PYRIMIDINES

Substance	*Max. ext. of 1 mg substance/ml*			*% of the purine or pyrimidine in the nucleic acid*	*Contribution to max. ext. of 100 mg nucleic acid per ml*		
	acid	*neutral*	*alkaline*		*acid*	*neutral*	*alkaline*
Adenine	101	105	93.5	9.5	960	998	888
Guanine	47	40	35	8.3	390	332	291
Cytosine	85	53	58	4.9	417	260	284
Thymine	58	59	40	8.1	470	478	324
				Total	2237 (1.08)	2068 (1.00)	1787 (0.86)

In all cases the maximal extinction values are used even if they do not always fall between 258–260 mμ. If the position of the maxima were taken into account this would mean a slight decrease in the calculated maximal extinction for the alkaline solution in comparison with the extinctions for neutral and acid solutions.

* The remark made in the earlier article[1] in reference to HEYROTH AND LOOFBOUROW[8] is incorrect.

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Samples of the four purines and pyrimidines were measured in the Beckman spectrophotometer at 10 points between pH 2 and 12. The results found were in very good agreement with Hotchkiss values. A mixture of the thus controlled substances was made with the proportions given in Table I. The absorption of the mixture was then measured at different pH values. The maximal extinction calculated from these measurements for the same purine and pyrimidine content as 100 mg nucleate per ml gives a value at pH 7 of 2070, the absorption at pH 2.8 was 1.05 times this, and that at pH 12.0, 0.85 times it. This means that a mixture of the four purines and pyrimidines gives an extinction in good agreement with the extinction calculated by direct addition of the extinction of the single components for all pH values.

The maximal extinction calculated for 100 mg nucleate per ml from the absorption measured on the nucleate preparations will be about 1950 in neutral solution. For the calculations of the nucleate concentrations from the nitrogen determinations the value N = 15.1% was used. The lower nitrogen percentage found by CHARGAFF *et al.*[6] would give an absorption of 100 mg nucleate/ml of about 2100.

Thus there is still good agreement between the calculated and the found absorption in neutral solutions of the nucleoprotein and nucleic acid preparations. But the ratio of the absorption at pH 7 to the absorption at pH 12 is found to be 1.32–1.33 instead of the expected 0.85–0.86. The fact that simple addition of the extinction values is no longer correct must be considered together with the picture of the position of the purines and pyrimidines in the nucleic acid which has been given by ASTBURY[15]. The distance between successive nucleotides piled one on top of the other in the nucleic acid is very small (the effective thickness of the nucleotide 3.4 A) and the purine and pyrimidine lie with their planes parallel to one another.

GULLAND, JORDAN AND TAYLOR[16] from their titrations on nucleic acid considered that liberation of enolic hydroxyl groups of guanine and thymine occurs between pH 8 and 12. They also supposed that there is a linkage between hydroxyl and amino groups by hydrogen bonding between nucleotides of the same or adjacent chains. These linkages are assumed to be broken in solutions of pH values higher than pH 10.9 (but also lower than pH 5.6).

It may be supposed that changes in the closely packed pile of purines and pyrimidines may account for the special effect of high pH on the ultraviolet absorption compared with a simple mixture of the purines and pyrimidines.

The increase in the maximal absorption with but a small shift in the wavelength of this maxima at high pH might serve as a useful guide to whether solutions contain polynucleotides or not.

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SUMMARY

Thymo-nucleoprotein prepared according to MIRSKY AND POLLISTER and nucleic acid prepared therefrom, following the method of GULLAND *et al.*, show a sharp increase in ultraviolet absorption at pH 11.0–11.3 by a factor of 1.32–1.33 times their absorption in neutral solution. The change in the wavelength of the maxima is very small.

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The absorption at pH values lower than 11 can be calculated by simple addition of the absorption of the four purines and pyrimidines in the nucleic acid. At a pH higher than 11 the special characteristics of the purines and pyrimidines *in situ* are supposed to give the increase in maxima absorption.

RÉSUMÉ

L'absorption dans l'ultraviolet de la thymonucléoprotéine préparée selon MIRSKY ET POLLISTER et de l'acide nucléique préparé à partir de cette nucléoprotéine suivant la méthode de GULLAND et collaborateurs augmentent brusquement à pH 11.0–11.3 et deviennent 1.32–1.33 fois plus grandes que l'absorption en solution neutre. La variation de la longueur d'onde des maxima est très peu importante.

On peut calculer l'absorption à des valeurs du pH inférieures à 11 par simple addition de l'absorption des quatre purines et pyrimidines de l'acide nucléique. A un pH supérieur à 11 les caractéristiques spéciales des purines et des pyrimidines *in situ* sont supposées provoquer l'augmentation de l'absorption maximale.

ZUSAMMENFASSUNG

Nach MIRSKY UND POLLISTER hergestelltes Thymonucleoprotein und daraus nach der Methode von GULLAND und Mitarbeitern hergestellte Nucleinsäure zeigen eine scharfe Zunahme der Ultraviolet-Absorption bei pH 11.0–11.3, welche 1.32–1.33 mal grösser wird als die Absorption in neutraler Lösung. Die Wellenlänge der Maxima wird nur wenig verändert.

Bei pH-Werten, welche niedriger sind als 11, kann die Absorption durch einfache Addition der Absorption der vier Purine und Pyrimidine in der Nucleinsäure berechnet werden. Bei höherem pH scheinen die besonderen Eigenschaften der Purine und Pyrimidine *in situ* die Zunahme der Absorption zu bewirken.

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