doit pas en être ainsi de toutes les réactions, mais on peut penser que nombre de réactions enzymatiques bénéficieraient d'un tel concours de circonstances favorables.

RÉSUMÉ

L'étude spectrographique des milieux d'oxydation du catéchol en milieu rigide (alcool à 96° à 100° K) après une intense et brève irradiation par la longueur d'onde qu'il absorbe en ultraviolet, montre l'existence d'une configuration transitionnelle que ses propriétés permettent d'assimiler au radical libre (semiquinone) correspondant.

L'action de la polyphénoloxydase pure de Champignon sur le catéchol dans les mêmes conditions conduit à des constatations expérimentales identiques.

BIBLIOGRAPHIE

[1] W. G. C. FORSYTH ET E. C. QUESNEL, *Biochim. Biophys. Acta*, 25 (1957) 155.
[2] S. BOUCHILLOUX ET S. LISSITZKY, *Bull. soc. chim. biol.*, 40 (1958) sous presse.
[3] G. N. LEWIS ET D. LIPKIN, *J. Am. Chem. Soc.*, 64 (1942) 2801.
[4] A. JABLONSKY, *Nature*, 151 (1933) 839.
[5] M. KASHA, *Chem. Revs.*, 41 (1947) 401.
[6] D. KERTESZ ET R. ZITO, *Nature*, 179 (1957) 1017.
[7] G. H. HOGEBOOM ET M. H. ADAMS, *J. Biol. Chem.*, 145 (1942) 275.
[8] L. MICHAELIS, *Transactions of the 4rd Conference of John Macy Foundation*, Vol. I, Academic Press, New York, 1950.

THE EFFECT OF CHYMOTRYPSIN ON THE MOLECULAR WEIGHT OF DESOXYRIBONUCLEIC ACID PREPARATIONS

J. HERMANS, JR.*

Laboratory for Inorganic and Physical Chemistry, University of Leiden (Netherlands)

(Received June 5th, 1958)

SUMMARY

Three preparations of DNA which did not dissolve in water were rendered soluble by treatment with chymotrypsin; the light-scattering molecular weights proved to be about 6 million. For eleven soluble preparations whose molecular weights ranged from 6 to 15 million, the molecular weights were likewise reduced to about 6 million.

Moreover, whereas the reciprocal of the reduced light scattering of several of the original samples decreased with increasing concentration of DNA, this abnormal behavior disappeared on treatment with chymotrypsin. The results are interpreted as meaning that a DNA unit exists with a molecular weight of about 6 million and that samples whose molecular weight is appreciably higher are aggregates of DNA molecules linked by protein.

* Present address: Chemistry Department, Cornell University, Ithaca, N.Y.

References p. 509.

INTRODUCTION

In a previous article[1] it was shown how upon heating in solution the molecular weight, as determined by light scattering, of one sample of desoxyribonucleic acid (DNA) decreased from $11 \cdot 10^6$ to $5 \cdot 10^6$. The value of $5 \cdot 10^6$ was thereafter remarkably resistant to further heating. The same was true of another sample with mol. wt. $\simeq 5 \cdot 10^6$, which confirms the results of RICE AND DOTY[2]. However, of a third sample, mol. wt. increased upon heating, although the radius of gyration dropped in a manner similar to that observed for the other samples.

In a critical study of several methods of preparing DNA, FRICK[3] has shown that in all DNA samples prepared by him some residual protein is present. This is certainly true for many, if not all the samples discussed by the present author in two previous articles[4,9], which describe the determination of viscosity, sedimentation and light scattering. Reference 4 contains a table of samples, which will be indicated as Table I-I, in which the origin, the method of preparation, the ratio N/P of nitrogen to phosphorus and the physical properties of a large number of DNA preparations are given. The ratio N/P which is known with an accuracy of about 3 % was larger than 1.70 for one half of the samples for which it was measured. The value calculated on the basis of the WATSON AND CRICK model[5] is 1.65.

As a working hypothesis the large variation of molecular weights as described in Table I, I may therefore be explained by assuming that, when the molecular weight is large, the samples contain molecules of DNA connected by protein links. Likewise the increase of mol. wt. occurring when heating sample 16 in solution would be due to the formation of such links. At this point we may refer to a study of ZUBAY AND DOTY[6], who showed that DNA and serum albumin agglomerate on heating.

To confirm this hypothesis we have investigated the action of chymotrypsin on DNA-samples. SHOOTER[7] has made an investigation of this kind: the distribution curves of the sedimentation constant S of some DNA-samples were investigated before and after treatment with chymotrypsin, and in some cases a shift was found to occur towards lower values of S. Since, however, the sedimentation constants of a number of DNA-samples show little variation[4,8], S is not a sensitive measure of small changes in structure. We have therefore restricted ourselves to light scattering and a few viscosity measurements.

METHODS AND MATERIALS

Preparations

Four insoluble samples, which were prepared at the Chester Beatty Institute (London) are described in Table I. Table II contains data concerning the soluble samples studied. The numbers in the first column of Table II are the same as in Table I-I. In the second column of Table I and in the third column of Table II the way in which the samples were prepared is listed by means of a code, the meaning of which is as follows:

The preparation was performed at

V = Strasbourg, Centre de Recherches sur les Macromolécules (C.R.M.), by R. VENDRELY

P = Strasbourg, C.R.M., by J. POUYET and the author

References p. 509.

L = London, Chester Beatty Institute
B = Leiden, Laboratory of Biochemistry.

The DNA was prepared by treating desoxyribonucleoprotein with

1 = concentrated NaCl[14]
3 = detergent[15]
4 = chymotrypsin[16]
5 = phenol–*p*-aminosalicylate[17].

The source was

a = calf thymus
b = fish sperm
d = rat thymus
e = rat spleen.

We wish to stress the fact that no evidence was found by us that any of these variables influences the physico-chemical properties of the samples in a systematic way[4]. We have therefore felt free to combine the data of all these samples.

Measurement

The measuring techniques have been described previously[4,9]. The molecular weight and the radius of gyration θ were derived from light scattering: if c is the polymer concentration, θ the angle of scattering, θ/E the intensity of the light scattered per ml of solution divided by the intensity of the incident light, r the distance from the scattering object to the observer, it is customary to introduce

$$R_\theta = r^2(I/E)(1+\cos^2\theta)^{-1}.$$

R_θ is a function of θ and c. The molecular weight and the radius of gyration are derived from a graphical representation of Kc/R_θ where K is a constant determined by the refractive index n and by the value of dn/dc. We followed the usual procedure of making "Zimm-plots", which show Kc/R_θ both as a function of $\sin^2(\theta/2)$ at a given value of c and as a function of c for a given angle θ. We find the molecular weight as the reciprocal of the intercept of the Zimm-plot with the (Kc/R_θ)-axis. The square of the radius of gyration is proportional to the slope of the tangent one can draw to the curve $\lim_{c=0}(Kc/R_\theta)$ as a function of $\sin^2(\theta/2)$ at the point where $\theta = 0$. (This tangent is drawn in Figs. 1 and 2). The radius of gyration is defined by $\varrho^2 = \int R^2 dm / \int dm$. The particle is divided in mass elements dm, R is the distance of each mass element to the center of gravity and the integrations are performed over the entire particle. ϱ is proportional to the dimensions of the particle when this has a given form. For a review on light scattering see ref.[18]. Its application to DNA is discussed by DOTY AND BUNCE[10].

Digestion

About 5 mg of chymotrypsin in a phosphate buffer of pH 7.5 was added to the gels which formed when the insoluble samples were brought into contact with water (30 mg of DNA in 100 ml of water) and the whole was left in a water bath of about 40° C during a period of 12 h. A grain of thymol was added to prevent the growth

References p. 509.

of microorganisms. These would produce sufficient amounts of acid to lower the pH to a value below 6, where chymotrypsin is no longer active. Essentially similar operations were carried out with solutions of samples from Table I-I, with the only difference that in a number of cases the solutions had already been used for light-scattering measurements. They had therefore been centrifuged and contained 1 mole NaCl/l.

RESULTS

The results are given in Tables I and II. Table I gives the molecular weight after digestion, Table II that before and after digestion. Furthermore, the radius of gyration and the dissymmetry of the scattered light $z_{45} = I_{45}/I_{135}$, where I stands for the intensity, are tabulated. z_W is a theoretical value of z_{45}. The way to obtain it is indicated below.

TABLE I

EFFECT OF CHYMOTRYPSIN ON FOUR INSOLUBLE DNA PREPARATIONS

Sample	*Code*	*Mol. wt.* $\times 10^{-6}$	$10^5\varrho$ *(cm)*	z_{45}	z_W	*N/P*
AB	L4d	7.6	2.15	3.8	3.8	1.8
CB	L3b	6.0	2.24	4.2	3.8	1.9
EB	L4e	6.3	1.86	3.2	3.7	2.1
DB	L3e		freeze-dried; does not dissolve			

TABLE II

LIGHT SCATTERING OF THYMUS DNA SAMPLES BEFORE AND AFTER TREATMENT WITH CHYMOTRYPSIN

No.	*Sample*	*Code*	$10^{-6} \times M$ *before*	$10^{-6} \times M$ *after*	$10^5\varrho$ *cm*	z_{45}	z_W	*N/P*
12	CV 69	V1a	15*	6.3	2.28	4.2	3.9	1.64
20	JJA	P1a	11	6.0	1.94	3.7	3.8	1.60
				4.3	2.50	3.5	3.3	
32	JC 1	B4a	11.6	6.5	2.14	3.8	3.8	1.77
33	JC 2	B4a	8.8	6.7	2.26	3.8	3.8	1.62
35	JCK 1	B5a	16	5.2	1.94	3.7	3.8	1.87
				5.7	2.10	4.1	3.8	
				5.3	2.02	4.2	3.8	
36	JCK 2	B5a	13	6.8	2.02	3.7	3.8	1.83
				5.8	2.14	4.5	3.8	
				5.4	1.95	3.7	3.8	
37	J 40	L3a	5.9	7.7	2.68	4.0	3.9	1.62
38	J 35	L3a	8.8	6.9	2.32	3.8	3.8	1.77
39	T 1	L3a	9.2	6.1	2.28	3.8	3.8	1.67
40	J 31	L2a	8.2	6.2	2.18	4.1	3.9	—
41	BB	L3a	11.5	2.9	1.94	3.65		—
24	AH 1a	B4a	18	14	1.98	4.1		1.75
25	AH 1b	B4a	36	20	—	—		1.84

* The mol. wt. of this sample was $4.6 \cdot 10^6$ originally (compare Table I-I). About a year later it was found that the mol. wt. had undergone an appreciable change.

References p. 509.

There is a curious observation we have not mentioned elsewhere. This is the occurrence of a negative slope of the Kc/R_θ versus c curves at constant θ in some of the Zimm plots. Fig. 1 shows the Zimm plot (compare[4]) of sample 36. The lines at constant θ have a negative slope. This is at variance with the behaviour of other polyelectrolytes at such high salt concentrations (1 M NaCl), and also with that of DNA as generally reported[10]. It is, however, representative of samples 23, 24, 25, 28, 32, 33, 35, 36, and 38 of Table I-I. Furthermore, solutions of these samples are much more turbid before centrifuging than after. In the cases we investigated this initial turbidity disappears upon the action of chymotrypsin on the solution. This is accompanied by a drop in mol. wt. and a disappearance of the negative slope.

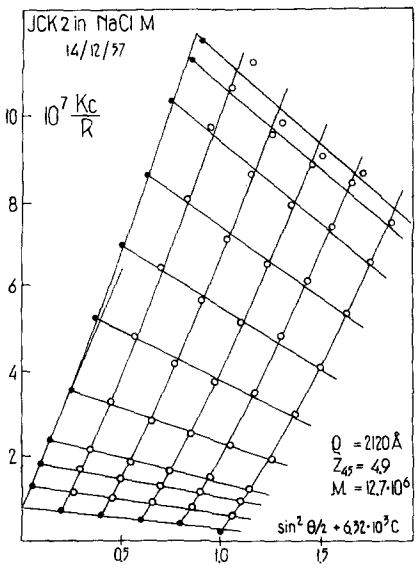

Fig. 1. Zimm plot of an untreated sample, showing negative slope of Kc/R_θ versus c at constant θ.

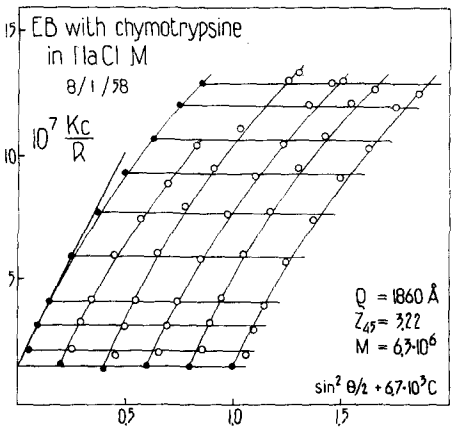

Fig. 2. Zimm plot of a sample treated with chymotrypsin.

Our conclusion regarding these samples is therefore that they contain aggregates of DNA molecules, presumably held together by protein residues. These aggregates need not, however, have such high sedimentation constants that they sediment completely during the one hour's centrifuging at 20,000 × g carried out before the light-scattering measurements. The negative slope is caused by the fact that more of these particles remain in solution when the viscosity is high, *i.e.* when the concentration is high. With increasing concentration we therefore have an increase of R, that is a decrease of Kc/R.

A close examination of the results as they are shown in Tables I and II reveals that in all cases investigated where mol. wt. $> 6 \cdot 10^6$ the action of chymotrypsin causes a drop in mol. wt. In only two cases is this drop not to a value close to $6 \cdot 10^6$ and in only one case does the mol. wt. drop much below this value. Only the freeze-dried sample apparently remains unaffected.

These experiments therefore finally bring agreement between the opinion of DOTY[11] that DNA consists of particles of approximately uniform molecular weight $6\text{–}8 \cdot 10^6$ and the findings of BROWN, MCEWEN AND PRATT[12] and of SADRON[13], who report much larger variations in molecular weight.

References p. 509.

In our previous articles[4,9] we have been able to demonstrate two relationships between the physico-chemical properties of DNA molecules.

In the first place[4] the intrinsic viscosity $[\eta]$ is roughly proportional to the square of the radius of gyration ϱ. Only in the case of sample 20 have we been able to measure the intrinsic viscosity after treatment with chymotrypsin. We found $[\eta] = 5300$ ml/g, a small change from the original value, 6000, which cannot be considered as very significant. As $\varrho = 2500$ Å for both treated and untreated sample 20, this means that the original DNA-structure is still intact. Solutions of the other samples containing chymotrypsin behaved poorly in the viscometer, and no values of $[\eta]$ have been obtained.

The second correlation observed is of a more complicated nature. It concerns the three quantities measured by light scattering, mol. wt., ϱ and z_{45}. At a given value of ϱ, z_{45} depends on the form of the solute particles. For certain models (*e.g.* rods, spheres, coils) the exact value of z_{45} for a given radius of gyration can be calculated. We were able to show[9] that for native DNA-samples the best agreement (though still not a perfect one) can be obtained by representing the DNA-molecule as a continuously curved or worm-like chain, the overall length of which is derived from the molecular weight with the aid of the WATSON AND CRICK[5] model. This model demands a molecular weight of 200 per Å. If the overall length and radius of gyration of a worm-like particle are known, one can calculate a theoretical value of z_{45}. This value, z_W, is included in Tables I and II. The agreement between calculated and measured values is on the whole the same as found for untreated samples[9].

It appears therefore safe to state that a DNA unit exists of molecular weight $6\text{–}8 \cdot 10^6$, in the form of a worm-like chain. Samples for which the molecular weight determined by light scattering, is considerably higher than this value (mol. wt. $> 9 \cdot 10^6$) contain particles consisting of two or more of these units linked by protein bridges.

REFERENCES

1 J. HERMANS AND A. M. FREUND, *J. Polymer Sci.*, 28 (1959) 229.
2 S. A. RICE AND P. DOTY, *J. Am. Chem. Soc.*, 79 (1957) 3937.
3 G. FRICK, *Biochim. Biophys. Acta*, 13 (1954) 51.
4 J. HERMANS AND J. J. HERMANS, *J. Phys. Chem.*, in the press.
5 J. D. WATSON AND F. H. C. CRICK, *Nature*, 171 (1953) 737.
6 G. ZUBAY AND P. DOTY, *Biochim. Biophys. Acta*, 23 (1957) 213.
7 K. V. SHOOTER, *Trans. Faraday Soc.*, 52 (1957) 247.
8 K. V. SHOOTER AND J. A. V. BUTLER, *Trans. Faraday Soc.*, 52 (1956) 734.
9 J. HERMANS, *J. Phys. Chem.*, in the press.
10 P. DOTY AND B. H. BUNCE, *J. Am. Chem. Soc.*, 74 (1952) 5029.
11 P. DOTY, *Symposium on Biocolloids, Gatlinburg*, 1956, p. 27.
12 G. L. BROWN, M. B. MCEWEN AND M. I. PRATT, *Nature*, 176 (1955) 161.
13 C. SADRON, *3rd Intern. Congr. Biochem., Brussels*, 1955, p. 210.
14 R. SIGNER AND N. SCHWANDER, *Helv. Chim. Acta*, 32 (1949) 853.
15 A. M. MARKO AND J. A. V. BUTLER, *J. Biol. Chem.*, 190 (1951) 165.
E. R. M. KAY, N. S. SIMMONS AND A. L. DOUNCE, *J. Am. Chem. Soc.*, 74 (1952) 1724.
16 J. A. V. BUTLER, D. W. F. JAMES AND B. E. CONWAY, *Trans. Faraday Soc.*, 50 (1955) 1612.
17 K. S. KIRBY, *Biochem. J.*, 66 (1957) 495.
18 C. SADRON, *Progress in Biophysics*, 3 (1953) 261–266.