A reasonable mechanism for the polymerization action of dextransucrase would be a termolecular process as postulated by Swain.[23] This author[23] was able to amplify an earlier suggestion by Lowry and Faulkner,[24] that in mutarotation α-D-glucose was subjected to a simultaneous attack by a nucleophilic and an electrophilic group. Later[25] it was demonstrated that of a series of hydroxypyridines only one the 2-hydroxypyridine contained the nucleophilic and electrophilic groups in the correct spatial arrangement for optimum catalysis of the mutarotation of tetra-*O*-methyl-α-D-glucopyranose. Such a situation might exist in dextransucrase where an imidazole and a carboxyl group are so situated in the protein matrix that they can effect a concerted attack on the glycosidic linkage of sucrose liberating a glucosyl group for the polymerization reaction. In this mechanism the rate-determining step would be the cleavage of the glycosidic linkage of the substrate.

The substrate specificity for dextransucrase is very rigid in that only sucrose has been shown to act as a glucosyl donor. It could be assumed, therefore, that apart from combination of the enzyme and substrate at a catalytic site, there must also be binding at a specificity site. Work is in progress to determine more about the nature of these two sites for this particular enzyme.

Acknowledgment.—The author is indebted to Dr. D. Dyer of this Laboratory for his advice and assistance in producing the enzyme.

MIDLAND, MICHIGAN

(23) C. G. Swain, THIS JOURNAL, **72**, 4578 (1950).
(24) T. M. Lowry and I. J. Faulkner, *J. Chem. Soc.*, **127**, 2883 (1925).
(25) C. G. Swain and J. F. Brown, THIS JOURNAL, **74**, 2538 (1952).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF ROCHESTER, SCHOOL OF MEDICINE AND DENTISTRY]

Composition and Properties of the Thymus Desoxyribonucleoprotein of Doty and Zubay[1]

BY ALEXANDER L. DOUNCE AND MARGUERITE O'CONNELL

RECEIVED JULY 10, 1957

In a recent communication to THIS JOURNAL, Doty and Zubay[2] reported the isolation of a nucleoprotein from calf thymus which contained approximately equal amounts of desoxyribonucleic acid (DNA) and protein, and which was thought possibly to be the principal structural component of chromosomes. Physiochemical studies of this material were reported. It was stated that gel formation by the nucleoprotein could be avoided by treating the material as specified in the communication.

In this Laboratory, however, we consider gel formation to be an indication of an intact desoxyribonucleoprotein, where the DNA is attached to the residual lipoprotein of the chromosomes, probably through covalent bonds.[3] Moreover it seemed likely to us that the method of isolation used by Doty and Zubay[1] would yield material approximating the isolated chromosomes of Mirsky in composition.[4-6]

We have therefore repeated the isolation of the material of Doty and Zubay[2] and have subjected this to analysis. In order to facilitate storage the material was dried from solution by the process of lyophilization. RNA and DNA were extracted from these samples with hot 5% TCA and determined by the method of Schneider, using the orcinol and diphenylamine reactions for color formation. Globulins were removed in cold 0.9% saline and precipitated with saturated ammonium sulfate. Water-soluble material (mainly inorganic) was determined by dissolving samples of the lyophilized nucleoprotein in water, drying at 105° and carefully washing the dried samples of the nucleoprotein with distilled water and drying them again at 105° for nine hours until the weight loss became negligible. Lipid was extracted from aliquots of the nucleoprotein in 25% ether–75% ethanol with a continuous Soxhlet extractor and then was isolated by dissolving in 30–60° petroleum ether. The material soluble in petroleum ether was determined by weighing after drying at 105° for nine hours. Histone was extracted from the lipid-free material with cold 0.2 *N* HCl and precipitated with concentrated NH_4OH on standing nine hours in the cold. The alcohol-precipitable histone was precipitated from the supernatant fluid by adding 3 volumes of 95% ethanol and allowing to stand for 2 to 3 days in the cold. The two histone samples were dried in the oven at 105°, carefully washed with distilled water to remove any salt and then redried at 105° before weighing. The residual protein was determined by first weighing the residue from lipid-extracted, histone-extracted material, after careful washing and drying to remove any salt that might be present. Then the weight contributed to the sample by RNA plus DNA was calculated and subtracted to give the weight of the residual protein. We find the composition to be approximately as given in Table I. Thus the material does in fact resemble Mirsky's isolated chromosomes in chemical composition.

The data that have been recalculated on the basis of material free of the water-soluble fraction

(1) We gratefully acknowledge the support of The National Cancer Institute, U. S. Public Health Service, Grant No. C-994, which has made this work possible.
(2) P. Doty and G. Zubay, THIS JOURNAL, **78**, 6207 (1956).
(3) (a) A. L. Dounce and K. J. Monty, *J. Biophys. Biochem. Cytol.*, **1**, 155 (1955). (b) K. J. Monty and A. L. Dounce, *J. Gen. Physiol.*, **41**, 595 (1958).
(4) A. E. Mirsky and A. W. Pollister, *ibid.*, **30**, 117 (1946).
(5) A. E. Mirsky and H. Ris, *ibid.*, **31**, 7 (1947).
(6) A. E. Mirsky and H. Ris, *ibid.*, **34**, 475 (1951).

TABLE I

PER CENT. OF CONSTITUENT (DRY WEIGHT)

Constituent	Material before dialysis against phosphate buffer	% based on material remaining after extracting sol. fraction of preceding column	Material after dialysis against phosphate buffer	% based on material remaining after extracting sol. fraction of preceding column
Material soluble in water after drying preparation at 105°	19.1	.....	28.4	.....
Globulin	Negligible	.....	Negligible	.....
Non-precipitable histone	17.0	21.0	13.4	18.7
Alcohol-precipitable histone	3.9	4.8	5.5	7.7
Total extractable histone	20.9	25.8	18.9	26.4
Residual protein	7.9	9.8	8.0	11.2
DNA	33.1	40.9	29.1	40.6
RNA	1.2	1.5	1.9	2.7
Lipid	2.2	2.7	2.2	3.1
Recovery	84.4%	106.5%	88.5%	110.4%

are all in reasonable agreement with those of Mirsky except for the total histone which is only two thirds as high as it was found to be by him. This might be accounted for in part by a failure on our part to precipitate the two histone fractions quantitatively. Our aim was to show that the preparation of Doty and Zubay is composed of a number of components rather than to offer a precise quantitative estimation of all of these components. It is also quite possible however that part of the histone is extracted by the Versene. In our experience whole liver nuclei isolated in aqueous media to which sodium chloride has not been added contain about twice as much histone as that reported by Mirsky for isolated liver chromosomes, so that losses of histone apparently can occur.

The fact that our residual protein fraction is not much higher than that of Mirsky leads us to believe that this fraction is not grossly contaminated with histone. The residual protein is necessary for the maintenance of chromosomal structure, if we interpret Mirsky's results and our own work on isolated nuclei correctly. The percentage of residual protein in thymus chromosomes is only 8.5 according to Mirsky, and if this amount were reduced very appreciably it is doubtful that chromosomal structure could be maintained. Therefore it seems very improbable to us that our residual protein could possibly consist mainly of unextracted histone, since microscopic examination of the material of Doty and Zubay during the centrifugations in saline showed that it was at first composed of whole nuclei and later chiefly of chromosomes and bundles of chromosomes with very definite morphology. After dissolving this material in water, nothing is done as far as we can ascertain that could lead to a loss in residual protein.

The fact that our analytical figures add up to less than 100% in the case of the original material and to slightly more than 100% after recalculating on the basis of material free of the water-soluble fraction might indicate that there is more firmly bound water present in the original material than in the material from which the water-soluble fraction has been removed. In any case precise recoveries cannot be expected in analyses of these sorts.

The magnitude of the water-soluble fraction obtained after drying the original material of Doty and Zubay at 105° may appear surprising, but much of this fraction seems to be composed of inorganic material. Some Versene may also be present. There are enough polar groups in DNA and histone to make the binding of considerable salt seem plausible when one considers that the first steps of the isolation procedure consist of repeated washings in saline–Versene solution; and the fact that the weight of the soluble fraction increases upon dialysis against phosphate buffer could be explained easily on the basis of exchange of phosphate ions for chloride ions. In any case this fraction is real and must be taken into account to explain the percentage of DNA in the preparation. The DNA content has been checked by phosphorus analysis and cannot be in error to any important extent.

In regard to the gelability of the material, it was found that if the washed sediment was suspended in water and stirred gently in the Waring Blendor by running the latter at greatly reduced speed, a strong elastic recoilable gel gradually formed as the material became hydrated. When this gel was subsequently stirred by means of a motor stirrer for intervals varying from one to three hours at about 2 to 3° the gel gradually became transformed to a viscous fluid characteristic of solutions of Na-DNA. On the other hand, if the blender was run at high speed in the original mixing, no gel formed. In this connection it is of interest to note that Mirsky found that deoxyribonucleoprotein gels could be destroyed by mixing in a high speed mixer,[7] and it is not unreasonable to suppose that long delicate nucleoprotein fibers could be reduced to much shorter segments by the mechanical action of the blendor, with the resultant destruction of the gel.

It is now clear that the action of DNA-ase I and very probably DNA-ase II can destroy the capacity of isolated cell nuclei to form gels in alkali or strong salt solutions without causing drastic depolymerizations of the DNA, and that a great increase in the extractability of the DNA accompanies this loss in gelability of the nuclei.[8] It is also clear that DNA-ase I action can occur during the isolation of cell nuclei in aqueous media.[8–10] How-

(7) A. E. Mirsky and A. W. Pollister, *Biol. Symposia*, **10**, 247 (1943).

(8) A. L. Dounce, M. P. O'Connell and K. J. Monty, *J. Biophys. Biochem. Cytol.*, **3**, 649 (1957).

(9) M. Goutier-Pirotte and A. Oth, *Biochim. Biophys. Acta*, **22**, 394 (1956).

(10) J. Rotherham, D. D. Schottlelius, J. L. Irvin and E. M. Irvin, *J. Biol. Chem.*, **223**, 817 (1956).

ever, the relatively high *p*H of the washing medium of Doty and Zubay as well as the presence of Versene make it seem unlikely that either DNA-ase I or II act to destroy the gel formed by slow mixing of the sediment with water in the Waring Blendor, during the process of stirring this gel with the motor stirrer in the ice-box. Nevertheless degelation does occur and this process is accompanied by a drop in *p*H of a few tenths of a *p*H unit, indicating a possible hydrolysis of some sort with the liberation of acid groups. It seems quite possible that such hydrolysis could be proteolytic in nature. Trypsin and chymotrypsin are known to destroy the gelability of liver cell nuclei isolated at *p*H 4.0 in dilute citric acid.[8]

Although we have not made extensive physiochemical studies of our material isolated by the method of Doty and Zubay, an examination of a sample by ultracentrifugation[11] indicated that it was similar in properties to the material isolated by them. Moreover the method of isolation is so simple that it would seem difficult to avoid obtaining their product.

In conclusion it seems obvious that the nucleoprotein of Doty and Zubay is a complex material consisting of several components, the main bulk of which appears to consist of DNA and histone. Since the latter two substances comprise macromolecules with opposite charges which would be expected to sediment together, and since the ultracentrifuge ordinarily does not separate DNA into different components, it does not seem surprising that the nucleoprotein material of Doty and Zubay should behave more or less as a single component in the ultracentrifuge, even in the absence of gel formation. It therefore seems surprising that these authors merely on the basis of particle size determination and N/P ratios can believe it likely that they have isolated a structural unit of the chromosome. Previous work on the composition and gelability of cell nuclei and chromosomes has not been mentioned by them, especially the role of the residual protein and the role of enzymes in destroying desoxyribonucleoprotein gels. The phenomenon of gel formation by the nucleoprotein has been lightly dismissed without justification on an experimental or theoretical basis. Neither has the possible role of the Waring Blendor in mechanically disrupting deoxyribonucleoprotein fibers been considered. Therefore the suggestion of Doty and Zubay that a fundamental unit of chromosomal structure has been isolated appears to be without sufficient experimental basis, and the concept that the formation of elastic recoilable gels by isolated nuclei and chromosomes is caused by a firm union of the DNA with the residual protein still appears valid to us.

As a final and very recent piece of evidence which we believe supports this concept indirectly but rather strongly, we refer to the work of Butler, *et al.*,[12] who found that the persistence of a small amount of strongly bound protein in isolated DNA can cause an abnormally high viscosity which can be reduced through the use of chymotrypsin.

(11) We wish to thank Dr. M. Schoenburg for subjecting a sample of the material in question to ultracentrifugation.

(12) J. A. V. Butler, D. M. Phillips and K. V. Shooter, *Arch. Biochem. Biophys.*, **71**, 423 (1957).

ROCHESTER, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Structures of Isomaltose and Gentiobiose

BY M. L. WOLFROM, A. THOMPSON[1] AND A. M. BROWNSTEIN[1]

RECEIVED NOVEMBER 27, 1957

An unequivocal proof of the structures of isomaltose and gentiobiose, based upon the identification of the hydrolytic fragments of their fully methylated aldonic acids (and methyl glycoside for isomaltose) is described. A method for isolating isomaltose and gentiobiose on a preparative basis from an acid reversion mixture of D-glucose is detailed.

The structure of isomaltose has been known for some time, with a considerable degree of certainty, to be 6-*O*-α-D-glucopyranosyl-D-glucose as shown by the periodate oxidation of the methyl glycoside,[2] isomaltitol,[3] and the free sugar,[4] as well as through the "acetobrominolysis" method of Jeanes, Wilham and Hilbert.[4] The proof of structure of gentiobiose rests upon its definitive synthesis by Helferich and co-workers[5]; the proof by methylation techniques[6] is incomplete.

We wish to report herein the preparation of methyl hepta-*O*-methyl-β-isomaltoside and its hydrolysis by acid to produce 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranose and 2,3,4-tri-*O*-methyl-D-glucose (identified as its aniline derivative). This proof is incomplete in that it assumes the presence of the pyranose ring in the reducing moiety. We wish therefore to describe rigid structural proofs for isomaltose and gentiobiose by the classical methylation procedure which Haworth, Loach and Long[7] applied to the definition of the structure of melibiose, 6-*O*-α-D-galactopyranosyl-D-glucose.

(1) Research Associate (A. T.) and Research Fellow (A. M. B.) of the Corn Industries Research Foundation. Preliminary work was carried out on this problem in this Laboratory by Mr. M. Inatome.

(2) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **71**, 125 (1949).

(3) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, *ibid.*, **74**, 1062 (1952).

(4) Allene Jeanes, C. A. Wilham, R. W. Jones, H. M. Tsuchiya and C. E. Rist, *ibid.*, **75**, 5911 (1953); Allene Jeanes, C. A. Wilham and G. E. Hilbert, *ibid.*, **75**, 3667 (1953).

(5) B. Helferich, K. Bäuerlein and F. Wiegand, *Ann.*, **447**, 27 (1926); B. Helferich and W. Klein, *ibid.*, **450**, 219 (1926).

(6) W. N. Haworth and B. Wylam, *J. Chem. Soc.*, **123**, 3120 (1923).

(7) W. N. Haworth, J. V. Loach and C. W. Long, *ibid.*, 3146 (1927).