mole fraction of residues with uncharged side-chains. It should therefore offer a more faithful model of protein behavior than previous polypeptides.

The tyrosine-containing copolymer $G_{51}L_{33}T_{16}$ offers some interesting contrasts to the alanine-containing copolymer $G_{42}L_{28}A_{30}$. Although the mole fraction of hydrophobic groups is less in $G_{51}L_{33}T_{16}$, the weight fraction of hydrophobic groups is higher and it is this hydrophobic burden that is probably decisive in favoring the β-conformation which gradually develops in these solutions. The rate of formation of the β-structure was found to increase at elevated temperatures and at high salt concentrations. This increased rate of formation of the β-structure with temperature, its culmination in gel formation, and its resolution by 8 M urea are all reminiscent of easily denatured proteins.

While the investigation of the properties of $G_{42}L_{28}A_{30}$ and $G_{51}L_{33}T_{16}$ is still in its early stages, it is clear that between them they exhibit in aqueous solution the whole range of characteristic protein properties deriving from interconversions among disordered coils, α-helical structures and β-conformations. Thus, these polypeptides represent a continual evolution toward the reproduction of the full range of the conformational properties of proteins in synthetic polymers that had its start with the display of the more limited features in poly-L-glutamic acid[17] and copoly-L-glutamic acid–L-lysine.[11]

Acknowledgment.—The authors would like to thank Mr. Peter Urnes for many helpful discussions concerning the results of the optical rotatory dispersion measurements.

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Use of Fully Deuteriated Algae Extracts for the Isolation of Nucleic Acids[1,2]

BY HENRY L. CRESPI, JULIUS MARMUR AND JOSEPH J. KATZ

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The composition of aqueous extracts of fully deuteriated algae is discussed and the utility of such extracts for the growth of fully deuteriated fastidious microörganisms is described. Fully deuteriated organisms grown in this way provide an excellent source of heavy DNA.

Introduction

Because essentially all of the hydrogen has been replaced by deuterium and because of a rich content of growth factors, extracts made from algae grown in D_2O have been found ideally suited for the growth of fully deuteriated, nutritionally-demanding microörganisms. These microörganisms serve as a very convenient source of isotopically altered compounds. In particular, a basal medium containing inorganic salts, and which is fortified by the addition of fully deuteriated carbohydrates and algae extract (Table I) has been successfully used to grow microörganisms (as well as their bacteriophage lysates) for the isolation of deoxyribonucleic acid (DNA). The medium will be referred to as DAEG. Fastidious organisms such as *Hemophilus influenzae* can be grown if the medium is supplemented with several vitamins as well as with diphosphopyridine nucleotide and hemin. Bacteriophage T7 will infect and lyse *Escherichia coli* grown in the medium described; moreover, it is possible to prepare bacteriophage lysates much more readily in DAEG than in the mineral salts–$N^{15}H_4Cl$–D_2O medium described previously.[3] Another advantage of the much more nutritionally-adequate DAEG medium in growing *E. coli* and other microörganisms is that the lag period and generation time in high concentrations of D_2O are greatly reduced.

(1) One of us (J. M.) was aided by a grant from the National Institutes of Health (RG-7985) and the National Science Foundation (G-13990).

(2) Based in part on work performed under the auspices of the U. S. Atomic Energy Commission.

(3) J. Marmur and C. L. Schildkraut, *Nature*, **189**, 636 (1961).

TABLE I

COMPOSITION OF MEDIUM FOR THE GROWTH OF ISOTOPICALLY SUBSTITUTED MICROÖRGANISMS

K_2HPO_4[a]	1.0 g.
KH_2PO_4[a]	0.25 g.
KCl	1.5 g.
NaCl	5.0 g.
Na_2SO_4	0.05 g.
Scenedesmus extract[b]	1.5 g.
Deuteriated sugars[c]	0.25 g.
D_2O (99+ per cent.)	1000 cc.

[a] The phosphate salts were dried down several times in D_2O before addition to the medium. The pD was adjusted before and during growth to approximately 7.5 with DCl or NaOD. [b] In the growth of bacteria for the isolation of DNA substituted with both D and N^{15}, *Chlorella* extract at 4.0 g./l. has been used. [c] Pure deuterio-glucose was used in the *Hemophilus* cultures.

Preparation and Characterization of Algae Extracts

Preparation.—The algae *Scenedesmus obliquus* and *Chlorella vulgaris* are grown in 99.6% D_2O in mass culture,[4] harvested by centrifugation, washed once with D_2O and stored at −20°. Periodically, 600 g. (wet weight) portions are thawed, thinned with D_2O and autoclaved at 15 pounds pressure (D_2O) for 15 minutes. Upon cooling, the gelatinous mass is centrifuged. The cell residue is washed once with D_2O, centrifuged and the two supernatant solutions pooled. After reducing the volume of the extract from *S. obliquus* to about 200 ml. by lyophilization, the whole turbid extract is clarified by centrifuging out at 18,000 g. for 20 minutes. The clear supernatant is then lyophilized to dryness. The extract from *C. vulgaris* is first lyophilized to complete dryness. The residue is taken up in D_2O, and the insoluble, gelatinous material that now appears is centrifuged out at 18,000 g. for 20 minutes. The residue is washed once, centrifuged and the pooled supernatant

(4) H. F. DaBoll, H. L. Crespi and J. J. Katz, in press.

TABLE II

ELEMENTAL ANALYSES

Extract	% C	% D	% N	% Ash	Inorganic P (mg./g.)	Total P (mg./g.)
SOD	31.3	11.6	6.39	23	37	62
CVD	33.1	11.9	2.57	22	16	26

Results

The DNA isolated from cells grown in DEAG medium is of high molecular weight and homogeneously substituted as determined by CsCl density gradient centrifugation.[10] It has been employed successfully in the study of DNA homologies of the *E. coli* bacteriophages[11] and more recently[12] in a similar study with several bacteriophages of *Bacillus subtilis*.

The DNA was isolated from the cells grown in deuteriated media (to titers of approximately 5×10^8) according to the method of Marmur.[13] If a growing culture is transferred to an H_2O medium, most of the cells begin to divide after a lag of several hours. DNA buoyant densities in CsCl were determined as described by Schildkraut, Marmur

TABLE III

COMPOSITION OF NON-IONIC FRACTIONS OF ALGAE EXTRACTS

Component	R_f	% of Non-ionic fraction SED[a]	% of Non-ionic fraction CED[b]	Benzidine color	Diphenylamine color
Glucose		15–30	15–25		
Sorbose		3–6	3–6		
Sucrose		1–5	None		
Ribose		Trace	Trace		
Unidentified	0.13 ± 0.01		Strong spot	Brown	Brown
Unidentified	.05 ± .01		Weak spot	Brown	....
Unidentified	.05 ± .005	Weak spot		Brown	Grey-blue
Unidentified	.07 ± .005	Strong spot		Brown	Brown
70% Ethanol insoluble		20	36		

[a] Xylose, raffinose, maltose, galactose, mannose were not found in SED extract. [b] Maltose, lactose, fucose, rhamnose, xylose, mannose were not found in CED extract.

solutions lyophilized to dryness. The *Scenedesmus* extract is designated SED, the *Chlorella* extract, CED.

Analysis.—Carbon, hydrogen and ash of the extracts were determined by combustion, nitrogen by the Dumas method, total phosphorus by the method of Kitson and Mellon.[5] Carbohydrates were determined by paper chromatography using butanol:water:acetic acid[6] as the developer. Both benzidine and diphenylamine spray reagents were used for identification. For the determination of purine and pyrimidine bases, the extract was digested in perchloric acid and chromatographed.[7] Inorganic phosphorus was determined by the method of Fiske and SubbaRow, as described by Umbreit, Burris and Stauffer.[8] Amino acids were estimated with the Beckman Spinco amino acid analyzer. The non-ionic material was separated from the ionic fraction of the extracts with MB-3 (Fisher Analytical Grade) mixed bed resin.

Characterization.—The final yield of D_2O-soluble extract is of the order of 15–20%, of the total dry weight of algae for both *Scenedesmus* and *Chlorella*. The insoluble, gelatinous material from *Chlorella* amounts to about 10% of the total dry weight. The results of elemental analyses of the soluble extracts are given in Table II. The high ash content of both extracts indicates the presence of considerable inorganic material. The lower nitrogen content of CED probably indicates a lesser amino acid content.

The *Scenedesmus* extract is composed of about 40% nonionic material while the *Chlorella* extract is about 60% nonionic. The composition of these nonionic fractions is given in Table III. Table IV lists those amino acids commonly derived from proteins that are present in SED. Also present in SED in more than trace amounts are phosphoserine, phosphoethanolamine, methionine sulfoxides (two peaks), γ-aminobutyric acid and ornithine. The approximate purine and pyrimidine content of SED (in mg./g.) is as follows: guanine, 2; adenine, 2; uracil, 3; cytosine, 1. Also present in SED extract in considerable amounts are at least three sugar phosphates, unidentified.

The sugar mixture that is also used in the culture media already has been characterized.[9] This mixture contains mainly glucose and mannose, with lesser amounts of at least four other sugars.

TABLE IV

AMINO ACIDS COMMONLY DERIVED FROM PROTEINS

Amino acid	Concentration in SED extract (mmole/g.)
Glycine	0.11
Alanine	.41
Valine	.075
Leucine	.05
Isoleucine	.023
Serine	.049
Threonine	.052
½ Cystine	.001
Methionine	.017
Aspartic acid	.044
Asparagine and glutamine[a]	.091
Glutamic	.01
Lysine	.04
Hydroxylysine	None
Arginine	0.02
Histidine	.032
Phenylalanine	.03
Tyrosine	.02
Tryptophane	.001
Proline	.058
Hydroxyproline	None

[a] These two components not separated.

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(12) R. Kallen and J. Marmur, unpublished results.

(13) J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961).

TABLE V

BUOYANT DENSITIES OF SUBSTITUTED AND NORMAL DNA IN CESIUM CHLORIDE

Organism	DNA density, g./cm.[3] Normal	deuterium substituted
Bacillus tiberius	1.694	1.737
Hemophilus influenzae	1.698	1.736
Bacillus subtilis	1.703	1.745[a]
Escherichia coli	1.710	1.751
E. coli phage T7	1.710	1.750
B. subtilis phage SP-8	1.743	1.787
[b]*Lemna gibba*	1.700	1.731

[a] The density of deuterium and N^{15} labelled *B. subtilis* DNA is 1.758 g./cc. [b] Grown in the presence of deuterioglucose[9] plus 50% D_2O, which is the maximum concentration compatible with rapid growth at this time.

and Doty.[14] Table V lists the densities of several representative samples of DNA isolated from bacteria, bacteriophages and *Lemna gibba* (duckweed). The buoyant density increase of the DNA due to the incorporation of non-exchangeable deuterium is approximately 0.042 ± 0.002 g./cc. This density increase is great enough under most circumstances to allow sufficient resolution in the interaction studies carried out between homologous DNA samples.[15] Denaturation of deuteriated *B. subtilis* DNA resulted in a density increase of 0.017 g./cc. By adding P^{32} to the DEAG medium described in Table I, it is possible to prepare nucleic acids whose fate can be followed in genetic and other studies. The ionic fraction from the acid hydrolysis of *C. vulgaris* cell wall[9] shows particular promise as a P^{32} labelling medium, as its phosphorus content is quite low. This ionic material can be substituted for the algae extracts described here.

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(14) C. L. Schildkraut, J. Marmur and P. Doty, *J. Mol. Biol.*, in press (1962).

(15) C. L. Schildkraut, J. Marmur and P. Doty, *ibid.*, **3**, 595 (1961)

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The Reaction of Imidoesters with Proteins and Related Small Molecules[1]

BY M. J. HUNTER[2] AND M. L. LUDWIG[3]

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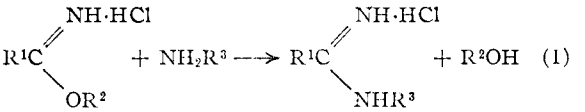
The possible use of imidoesters as group specific reagents for the modification of protein amino groups was investigated. By the use of model compounds it was established that, of the reactive groups present in proteins, only the amino group reacted with imidoesters in aqueous solvents. Balance studies of the reaction between glycine and methyl benzimidate at *p*H 9.5 indicated that the amino group was converted to a single reaction product, the corresponding monosubstituted amidine. The rates of reaction of methyl benzimidate and methyl acetimidate with glycyl-glycine and ϵ-NH_2 caproic acid were measured over the *p*H range 7–11. The rate was found to be strongly *p*H-dependent, passing through a maximum whose position on the *p*H scale was a function of the nature of the amine and the imidoester. Insulin–imidoester reactions were performed at room temperature and at *p*H values >7 but <10. Reaction with excess reagent resulted in a complete loss of the protein α- and ϵ-amino groups, the lysyl peptide bond being resistant to trypsin after reaction. Both the lysyl and the N-terminal glycyl residue appeared to have been completely converted to the corresponding amidine, but some minor side-reaction at or near the N-terminal phenylalanyl residue occurred during the reaction of the protein with aliphatic imidoesters. In general, it appears that the reaction of proteins with imidoesters does afford a useful means for the specific modification of protein amino groups to any of a variety of amidino groups under relatively mild conditions.

Introduction

Of the reagents in general use for the modification of proteins, few react with only one type of side-chain residue. Moreover, many of the available reagents yield derivatives whose solubility in water is very limited, largely due to the conversion of charged protein reactive groups to uncharged substituted groups. The search for a new group-specific reagent which would not *per se* alter the charge on the protein led to a re-investigation of the formation of amidines by the well-known imidoester–amine reaction



The reaction is formally analogous to that between O-methylisourea and amines.[4]

The preparation and reactions of imidoesters were studied many years ago by Pinner[5] who referred to them as "imidoäther" (iminoethers). More recent reviews have proposed to name these compounds imidates, or imidoates, as they are esters of the hypothetical imidic acids, RC(=NH)OH.[6,7] In this paper the class of compounds will be referred to as imidoesters; specific compounds will be called imidates, *viz.*, methyl benzimidate.

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(3) Department of Chemistry, Harvard University, Cambridge, Mass. The work described in this paper was carried out while this author was the recipient of a post-doctoral fellowship (#CF-6611) from the National Cancer Institute.

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