Formation of Particulate Ribonucleoprotein with Enzymatic Activities *in vitro*

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During the purification of lactic dehydrogenase from baker's yeast [1] a particulate ribonucleoprotein was observed to be formed in a soluble preparation of proteins and ribonucleic acid. The solution was obtained from the mechanically extracted cells by acetone precipitation, calcium phosphate adsorption/elution and ammonium sulfate precipitation. The nucleoprotein precipitated on storage at 6°C from the saltfree solution which had been concentrated by lyophilization.

The precipitate contained about equal amounts of nucleic acid and protein. Adenine, guanine, cytosine and uracil were all present. The nucleoprotein (nucleic acid and protein) was insoluble in water and in different salt solutions (*e. g.* 1 M sodium chloride), but dissolved in 0.04 M sodium hydroxide. In some preparations the particles settled easily in a low centrifugal field, in others a clear solution was not obtained even after 30 min centrifugation at 30 000 × *g*.

The original solution contained 10 % ribonucleic acid, 5—10 % each of lactic-, malic-, alcohol- and triosephosphate dehydrogenase, and had proteolytic and nucleolytic activities. The well-washed particles had malic dehydrogenase activity. None of the other dehydrogenases were present. The particles digested the particulate ribonucleic acid. Thus, on incubation in phosphate buffer purine and pyrimidine material appeared in the supernatent. This material was not liberated by boiling of the particulate ribonucleoprotein.

Malic dehydrogenase was measured spectrophotometrically at 340 mμ, with oxalacetic acid and DPNH at pH 8.4, with malic acid and DPN at pH 9.6. The rate was 40 μmoles oxalacetic acid reduced per mg particulate protein per hour. Highly purified malic dehydrogenase has been obtained from the original ammonium sulfate precipitate by zone electrophoresis on cellulose. This soluble preparation did not contain ribonucleic acid and had an activity of 8 500 μmoles oxalacetic acid reduced per mg protein per hour.

The results indicate that particulate ribonucleoproteins isolated from bacterial cells may not necessarily represent original particles of the cell. Particles may be formed in a haphazard manner during extraction and purification.

1. Nygaard, A. P. *Biochim. et Biophys. Acta. In press.*

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Standard Deviations of Interatomic Distances in Completely Deuterated Benzene

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In a previous communication the calculation of standard deviations of interatomic distances in the benzene molecule has been reported [1]. The calculated values have been compared with values obtained from electron diffraction data [2].

Similar calculations from spectroscopic data have been performed for the benzene-d_6 molecule with the result given in Table 1. The experimental fundamental

Table 1. Standard deviations for interatomic distances in benzene-d_6.

Distance	Calculated standard deviations in Å units $T = 0$	273.16	298.16	323.16
C_1—D_1	0.0660	0.0660	0.0660	0.0660
C_2—D_1	0.0889	0.0895	0.0898	0.0902
C_3—D_1	0.0835	0.0845	0.0848	0.0952
C_4—D_1	0.0821	0.0834	0.0839	0.0844
C_1—C_2	0.0455	0.0456	0.0457	0.0458
C_1—C_3	0.0529	0.0539	0.0543	0.0546
C_1—C_4	0.0570	0.0587	0.0593	0.0599
D_1—D_2	0.1277	0.1290	0.1296	0.1303
D_1—D_3	0.1095	0.1107	0.1112	0.1117
D_1—D_4	0.1012	0.1024	0.1028	0.1033

frequencies given by Brodersen and Langseth [3] have been applied. To the knowledge of the author, the molecule has not been investigated by electron diffraction.

1. Cyvin, S. J. *Acta Chem. Scand.* **11** (1957) 1499.

2. Bastiansen, O. and Cyvin, S. J. *Nature* **180** (1957) 980.
3. Brodersen, S. and Langseth, A. *Kgl. Danske Videnskab. Selskab, Mat. Fys. Skrifter* **1** (1956) No. 1.

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Two New Naturally Occurring Methoxyl-Containing Carotenoids

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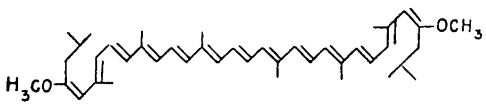
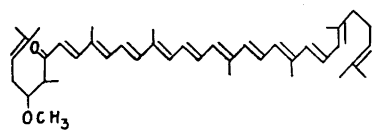
Methoxyl-containing carotenoids have so far been restricted to the photosynthetic bacteria. Carotenoids hitherto known to contain methoxyl groups are:

1) Spirilloxanthin, isolated by van Niel *et al.*[1] in 1935 from old cultures of *Rhodospirillum rubrum*, and shown by comparision of analythical data [2] to be identical with rhodoviolascin, isolated the same year by Karrer *et al.*[3] from mixed cultures of *Rhodovibrio* species. Karrer *et al.* have proposed the formula for spirilloxanthin [4].



2) Pigment Y (Yellow) and Pigment R (Red), isolated from *Rhodopseudomonas spheroides*, and both shown by Goodwin *et al.*[5] to contain one methoxyl group. For Pigment R Goodwin *et al.* have proposed the formula



and renamed it spheroidenone [5].

Kinetic experiments performed by Stanier *et al*[6]. to establish the route of carotenoid synthesis in the photosynthetic, nonsulphur purple bacterium *Rhodospirillum rubrum*, showed that the following transformation occurs quantitatively during growth:

lycopene → P481 → spirilloxanthin
OH-lycopene → OH-P481 → OH-spirilloxanthin

The structures of the carotenoids P481 and OH-P481 are not known. The designation Pigment 481 was given by Goodwin *et al.*[7] from the main absorption band in petroleum ether.

During endogenous carotenoid synthesis (anaerobically in light) washed cells of *Rhodospirillum rubrum* previously grown in the presence of diphenylamine, produced considerable amounts of P481 and OH-P481, whereas only a limited synthesis of spirilloxanthin normally was observed. This observation led to the assumption that P481 and OH-P481 were carotenoids with the same C_{40} skeleton as lycopene, and that lack of intracellular methyl donators limited the endogenous spirilloxanthin synthesis [8].

In the investigation now reported the presence of methoxyl groups in crystalline, chromatographically pure P481 and OH-P481 was indicated by IR-absorption bands at 1 078 cm^{-1} (KBr discs).

Quantitative methoxyl determinations were carried out according to the modified method of Inglis [8], and gave the following results:

Carotenoid	% OCH_3 found	% OCH_3 calculated	based on formula
P481	5.51	5.48	$C_{40}H_{55}(OCH_3)$
OH-P481	5.33	5.31	$C_{40}H_{54}(OH)(OCH_3)$

These results establish the presence of one methoxyl group in both P481 and OH-P481.

The fact that P481 and OH-P481 are C_{41} compounds is not in accordance with our previous suggestion. However, this finding fits well the role that has been ascribed to these two carotenoids as biosynthetic intermediates between lycopene ($C_{40}H_{56}$) and spirilloxanthin ($C_{42}H_{60}O_2$)[6].

Work on the chemical structure of P481 and OH-P481 is in progress.

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