

filaments similar to the interaction between actin and myosin filaments during contraction of vertebrate striated muscle. If this is so, then the foregoing results provide further support for the sliding filament hypothesis proposed for vertebrate striated muscle^{8,9}. The actin-myosin interaction might involve either long-range (for example, electrostatic) forces¹⁰, or a cycling system, with most individual actin and myosin molecules in their 'resting' configurations at any given time during contraction¹¹.

We thank Sir John Randall for providing facilities for this work, Dr. J. Lowy and Dr. Jean Hanson for their advice, and Mr. Z. Gabor for photographic assistance.

B. M. MILLMAN
G. F. ELLIOTT

Medical Research Council,
Biophysics Research Unit,
King's College,
26-29 Drury Lane,
London, W.C.2.

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BIOCHEMISTRY

Thymidine Diphosphate Nucleotides as Substrates in the Sucrose Synthetase Reaction

THE UDP-glucose : D-fructose 2-glucosyltransferase (sucrose synthetase) is considered to be responsible for much of the synthesis of sucrose in plants¹. Recently the important role of this enzyme in the degradation of sucrose in plant storage tissues was pointed out². Other nucleotides, notably ADP, can also serve as glucosyl acceptors in this system^{3,4}. Furthermore, tracer experiments with carbon-14 have shown that ADP-glucose formation from sucrose and ADP is a channel for the transfer of glucose from sucrose to starch^{4,5}, and that TDP-glucose is a substrate for sucrose synthesis in a crude enzyme preparation obtained from wheat endosperm³.

A detailed quantitative study of the specificity of this enzyme to nucleoside diphosphates serving as glucosyl acceptors has now been made using a highly purified sucrose synthetase from sugar beet root⁶, which is free of invertase and phosphatase activities. The results presented in Table 1 show that TDP is a very efficient acceptor whereas ADP is much less effective in this case than with the sweet corn enzyme system⁴. Chromatographic analysis⁷ of reaction systems containing UDP, TDP and ADP, as described in Table 1, indicated the appearance of new nucleotide components which behaved in the same way as authentic UDP-glucose, TDP-glucose and ADP-glucose, respectively.

Table 1. EFFECT OF NUCLEOSIDE DIPHOSPHATES ON SUCROSE CLEAVAGE BY SUCROSE SYNTHETASE

Nucleotide	Fructose liberated (μmoles/ml./h)	Relative activity
UDP	1.29	100
TDP	0.67	52
ADP	0.20	16
CDP	0.15	12
GDP	0.08	6
None	0.02	—

The reaction mixture contained: *tris*, 25 mM; sodium acetate, 20 mM; mono-sodium phosphate, 20 mM (pH 6.0); sodium fluoride, 20 mM; sucrose, 200 mM; EDTA, 1.5 mM; nucleotide, 2.5 mM; purified enzyme, 22 μg protein/ml.; and bovine serum albumin, 50 μg/ml. Incubation was at 37°.

The amount of fructose liberated was measured as described previously⁸.

From a reaction mixture similar to that described in Table 1, and containing TDP as the nucleotide, the new sugar nucleotide component was quantitatively isolated by paper chromatography, analysed chemically and enzymatically, and shown to be TDP-D-glucose, using the chemical and enzymatic procedures already described⁸. A reaction mixture which contained *tris* buffer, 120 mM at pH 7.2; fructose, 60 mM; EDTA, 6 mM; sodium fluoride, 30 mM; TDP-glucose, 6 mM, and purified enzyme, 144 μg/ml., contained, after incubation for 3 h at 37°, about 3 mM disaccharide which was identified as sucrose by paper chromatography, specific colour reactions and enzymatically².

Kinetic investigations² have clearly shown that UDP and TDP as well as UDP-glucose and TDP-glucose are competing substrates for the same site of the transglucosylase. The V_{max} value for sucrose synthesis at pH 7.2 with UDP-glucose as the substrate was 3.7 times higher than that observed for TDP-glucose. The V_{max} value for sucrose cleavage at pH 6.0 using UDP as the glucosyl acceptor was 1.7 times higher than that observed when TDP was used. The apparent K_m values obtained for the uridine and thymidine nucleotides were of the same order of magnitude (Table 2).

Table 2. APPARENT K_m VALUES FOR THE URIDINE AND THYMIDINE NUCLEOTIDES

Substrate	pH of assay	K_m (M)
UDP	6.0	7.7×10^{-6}
TDP	6.0	9.4×10^{-6}
UDP-glucose	7.2	2.7×10^{-4}
TDP-glucose	7.2	9.5×10^{-5}

Sucrose cleavage was followed by measuring the appearance of free fructose in a system containing 0.25 M sucrose and varying concentrations of nucleoside diphosphate. Sucrose synthesis was assayed by measuring the appearance of UDP, or of free glucose liberated by addition of yeast invertase, in a reaction mixture containing 10 mM fructose and varying concentrations of sugar nucleotides. Analyses were carried out as described elsewhere².

TDP-glucose is an intermediate in the biosynthesis of TDP-L-rhamnose and TDP-D-galactose, which serve as glycosyl donors in the biosynthesis of complex saccharides^{9,10}. It is thus possible that formation of TDP-glucose by the sucrose synthetase of plant storage tissues, where sucrose is found in high concentration, is a significant pathway for the supply of glycosyls to various biosynthetic reactions occurring in these tissues. Recent experiments in our laboratory have shown the presence of several thymidine diphosphate sugar derivatives which occur naturally among the soluble nucleotides obtained from sugar beet roots.

This work was supported by research grant *FG-IS-141* from the U.S. Department of Agriculture.

YORAM MILNER
GAD AVIGAD

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Hebrew University of Jerusalem,
Israel.

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Optical Rotatory Dispersion of Cytochrome c Phospholipid Complexes

PHOSPHOLIPIDS are primary structural materials of the mitochondrial membrane, and numerous observations implicate them in the function of cytochrome c (ref. 1). Thus, when bound in the mitochondrial membrane, cytochrome c is not easily extractable and permits higher rates of electron transport than does added cytochrome c