# DESOXYADENYLIC ACID AS ACCEPTOR FOR HIGH ENERGY PHOSPHATE*

by

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While the participation of adenosine-5-phosphate (AMP) and its derivatives in phosphate transfer reactions has been known for many years, and more recently uridine-5'-diphosphate and 2-aminoadenosine have been shown to be phosphorylated to the corresponding triphosphates[1,2] which are analogs of adenosine triphosphate (ATP), no participation of desoxyribose nucleotides in phosphate transfer reactions has been reported. In the course of an investigation of the metabolic role of desoxyribose nucleotides we have found that 2'-desoxyadenosine-5'-phosphate (DAMP) can be phosphorylated to the corresponding di- and triphosphates.

At first various combinations of purified enzymes[2,3] (pyruvate phosphokinase, myokinase and lactic dehydrogenase) were used which catalyze the formation of adenosine diphosphate (ADP) and ATP from AMP and phosphoenol pyruvate (PEPA). Since the direct transfer of phosphate from PEPA to AMP is minor or absent in the system, a small amount of ADP must be present initially. The oxidation of reduced diphosphopyridine nucleotide (DPNH) by pyruvate liberated from PEPA is a measure of the phosphate transfer. In the absence of acceptor other than ADP there was a correspondingly small oxidation of DPNH, while the subsequent addition of AMP or DAMP led to complete oxidation of the DPNH. When incubated mixtures to which DAMP had been added were analyzed by paper chromatography[4] with the $Na_2HPO_4$-*iso*amyl alcohol solvent, two principal bands were observed, one with the same $R_F$ as AMP and representing the unused DAMP, and one with an $R_F$ similar to ADP. Chromatograms of control incubation mixtures demonstrated that the catalytic amount (0.03 $\mu M$) of ADP added to the reaction mixtures was barely detectable whereas the spot with the $R_F$ corresponding to ADP appeared to contain about half the initial amount of DAMP added (0.9 $\mu M$). This strongly suggested the formation of 2'-desoxyadenosine diphosphate (DADP), probably by the action of myokinase, and it seemed likely that the corresponding desoxy ATP (DATP) might also be formed.

Large scale experiments with the purified enzymes, designed to prepare the presumed desoxyadenosine polyphosphates in quantity, have been unsuccessful thus far and consequently a different system was employed. Mitochondria were prepared from homogenized rabbit kidney cortex, by the method described by SCHNEIDER[5] for rat liver. After washing, the mitochondria were suspended in 0.25 $M$ sucrose and fortified with appropriate amounts of ATP, DPN, cytochrome C, $MgCl_2$, NaF and phosphate buffer[5,6,7]. This preparation was used in all subsequent experiments, with potassium glutamate as the oxidizable substrate. In several experiments determination of inorganic phosphate uptake and P/O ratios demonstrated that DAMP was almost as effective a phosphate acceptor as AMP, although the P/O ratios were never as high as those obtained when P/O ratios were measured using hexokinase and glucose as the acceptor system.

Net synthesis of polyphosphates from AMP and DAMP was accomplished as follows:

*Experiment I.* The mixture, final volume 15 ml, contained 5 ml enzyme solution (corresponding to 4.4 grams wet weight of kidney cortex, and containing 5 $\mu M$ ATP), 250 $\mu M$ inorganic phosphate and 125 $\mu M$ AMP. Incubation was at 37° in a 50 ml Warburg flask. After 50 minutes the mixture was transferred to a large test tube and placed in a boiling water bath for 5 minutes, then chilled in ice, filtered, an aliquot of this filtrate was diluted with 9 volumes of 6% perchloric acid and filtered again. Analysis for inorganic phosphate and 10 minute acid-labile phosphate[8] showed that 200 $\mu M$ of inorganic phosphate had been esterified and 54 $\mu M$ of acid-labile phosphate were present. The remainder of the mixture was absorbed on a column of Dowex-1-chloride, 200–400 mesh, $\pi$ $cm^2$ × 10 cm, and eluted according to COHN AND CARTER[9]. Eluates corresponding to ADP and ATP were neutralized and analyzed. The results are shown in Table I. There can be little doubt concerning the identity of the ADP and ATP fractions.

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TABLE I

ANALYSES OF ADP AND ATP FRACTIONS FROM EXPERIMENT I

| | *"ADP Fraction"* | | *"ATP Fraction"* | |
|---|---|---|---|---|
| | *μM/ml* | *Ratio (referred to adenine)* | *μM/ml* | *Ratio* |
| Adenine* | 0.0899 | 1 | 0.166 | 1 |
| Total phosphate** | 0.174 | 1.93 | 0.519 | 3.2 |
| Acid-labile phosphate | 0.075 | 0.84 | 0.311 | 1.82 |
| Ribose*** | 0.0945 | 1.05 | 0.173 | 1.04 |
| Volume | 202 ml | | 115 ml | |

On the basis of the adenine content, these figures represent 18 $\mu M$ ADP and 19 $\mu M$ ATP.

* Calculated from the molecular extinction coefficient of adenosine = $1.48 \cdot 10^4$ $cm^2$/mole.
** Inorganic phosphate was absent, as determined by the method of LOWRY AND LOPEZ[10].
*** By the method of MEJBAUM[11].

*Experiment II.* This experiment was identical with experiment I, except that 45 $\mu M$ DAMP were present, and AMP omitted. Phosphate analysis at the end of the incubation showed that 94 $\mu M$ of inorganic phosphate had been esterified. Acid-labile phosphate measurements are difficult to interpret since the DAMP is itself acid-labile. The deproteinized incubation mixture was analyzed by ion exchange exactly as in experiment I. Measurement of ultraviolet absorption showed that the "ADP" fraction (163 ml) contained 10.1 $\mu M$, and the "ATP" fraction (137 ml) contained 8.1 $\mu M$ of adenine derivative, and chemical analysis showed that appropriate amounts of phosphate were present. Both solutions were lyophilized, and the resulting powders dissolved in water, final volume 10 ml.

The analyses of these solutions are shown in Table II. The ratio total sugar/purine which should be 1:1 is low in both fractions, but this is not surprising in view of the fact that the colorimetric tests are markedly affected by changes in the conditions under which they are carried out[13]. It should be pointed out that the "ADP" and "ATP" fractions were respectively 0.5 $M$ and 2.9 $M$ in NaCl, and that during the analysis for desoxyribose it was apparent that the color developed was somewhat different from the standard. On the basis of purine analyses the ADP fraction contains 8.9 $\mu M$ and the ATP fraction 7.4 $\mu M$. If one assumes that the analyses for desoxyribose gave values which were too low rather than too high, then these must be at least 5.1 $\mu M$ DADP and 3.1 $\mu M$ DATP in these fractions.

TABLE II

ANALYSES OF CONCENTRATED ADP AND ATP FRACTIONS FROM EXPERIMENT II

| | *"ADP Fraction"* | | *"ATP Fraction"* | |
|---|---|---|---|---|
| | *μM/ml* | *Ratio (referred to adenine)* | *μM/ml* | *Ratio* |
| Adenine | 0.89 | 1 | 0.74 | 1 |
| Total phosphate* | 1.74 | 1.96 | 2.14 | 2.89 |
| Desoxyribose** | 0.51 | 0.57 | 0.31 | 0.42 |
| Ribose | 0.23 | 0.26 | 0.21 | 0.28 |
| Total sugar | 0.74 | 0.83 | 0.52 | 0.70 |
| Volume | 10 ml | | 10 ml | |

* Inorganic phosphate was absent. Acid-labile phosphate was not determined.
** By the procedure of STUMPF[12].

It is concluded that the phosphate transferring enzymes of muscle and of kidney mitochondria can utilize DAMP in place of AMP. Current experiments are designed to improve the yield and purity of DADP and DATP in order that their chemical and biological properties may be studied.

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# REVERSIBLE TRANSGLUCOSIDATION OF ISOMALTOSE*

by

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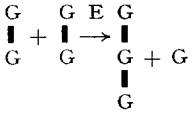
In a previous publication[1] it was shown that the transglucosidase of *Aspergillus oryzae* converted maltose to glucose, isomaltose (dextrobiose), panose and dextrotriose. The persistence of the α-1,6-glucosyl oligosaccharides in the reaction mixture indicated an equilibrium state and a reversibility of enzyme action. Experiments with radioactive tracers showed that the enzyme did not act reversibly on maltose. In this communication evidence is presented for the reversible transglucosidation of isomaltose by the *A. oryzae* transglucosidase.

Isomaltose (dextrobiose) and dextrotriose, the di- and trisaccharides of the dextran series of oligosaccharides, were isolated from a partial acid hydrolysate of dextran. Four mg of pure isomaltose (dextrobiose) was dissolved in 0.05 ml of water and mixed with 0.05 ml of a transglucosidase solution from *Aspergillus oryzae*[1]. An aliquot of 0.01 ml of this mixture was placed on a paper chromatogram and heated for 5 minutes at 100° C to arrest enzyme action. Subsequent samples were obtained at 12, 48 and 96 hours. The reducing compounds in these aliquots were separated and identified by paper chromatographic methods[2]. Initially, isomaltose was the only reducing compound in the reaction mixture; at 12 hours glucose and dextrotriose appeared in low concentrations; at 48 and 96 hours the concentrations of the glucose and dextrotriose increased while the concentration of isomaltose decreased. Since transglucosidase transfers glucose residues from a substrate to the 6-position of glucosyl cosubstrates[1,3] the action of the enzyme on isomaltose would proceed by the mechanism shown in the accompanying equation. (G, E and ▮ represent glucose unit, enzyme molecule and an α-1,6-glucosidic bond, respectively).



The reversibility of this reaction was tested with radioactive glucose and dextrotriose as substrates for the enzyme. One mg of $^{14}$C-glucose (total activity 26,000 cpm) and 6 mg of dextrotriose

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