Versuche mit Glucose, die an C_1 bzw. C_6 mit ^{14}C markiert war, beweisen nun die von uns angenommene Verschiebung des Glucose-Abbaues in Hefezellen. Man sieht aus Tabelle I, dass der Zusatz von NH_4^+ zu Glucose oxydierender Hefe die $^{14}CO_2$-Freisetzung um das 2.5 fache zugunsten von [1-^{14}C]Glucose verschiebt. Dies spricht eindeutig für eine Intensivierung des Glucose-Abbaues über den oxydativen Pentosephosphatcyclus nach Zusatz von Ammoniumsalzen. Die durch NH_4^+-Zusatz ausgelöste Oxydation von TPNH zu TPN findet wahrscheinlich durch 2 Mechanismen statt: (a) Reduktive Aminierung von α-Ketoglutarat zu Glutaminsäure, (b) Wasserstoffübertragung von TPNH auf DPN durch Zusammenwirken der in Hefe nachgewiesenen DPN-spezifischen und TPN-spezifischen Glutaminsäuredehydrogenasen[9]. Hefezellen, die in Phosphatpuffer glucose oxydieren, erhalten mit dem Angebot von Ammoniumsalzen die entscheidende stoffliche Voraussetzung für Wachstum und Zellvermehrung. Deshalb liegt in der nach Ammonium-Zusatz einsetzenden vermehrten Produktion von Pentosephosphaten aus Glucose ein "sinnvoller" Mechanismus vor: Pentosephosphate werden zur Synthese von Nucleinsäuren benötigt und stellen damit wichtige, für Wachstum und Zellvermehrung benötigte, Stoffwechselzwischenprodukte dar.

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Physiologisch-Chemisches Institut der Universität, Freiburg (Deutschland)

HELMUT HOLZER
IRENE WITT

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Activation of methionine for transmethylation
IV. The failure of 3,5′-cycloadenosine to replace adenosine triphosphate*

The enzymic activation of methionine for transmethylation is known to involve the formation of a sulfonium compound[1], (—)-S-adenosyl-L-methionine**, by a reaction

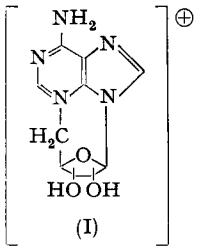
Abbreviations: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate.

* Paper No. 17 in a series on enzymic mechanisms in transmethylation.

** In the term (—)-S-adenosyl-L-methionine the L refers to the configuration of the α-amino carbon atom while the (—)refers to the contribution of the asymmetric sulfonium center to the net rotation of the molecule. This terminology is based upon the finding that only one sulfonium diastereoisomer is formed enzymically and is active as a transalkylating agent with several enzyme systems[2].

in which the terminal phosphate group of ATP is released as orthophosphate and the proximal two are released as pyrophosphate[3,4].

Earlier studies on the mechanism of this reaction led to the suggestion that it might proceed stepwise with the intermediate formation of a 3,5′-*cyclo*adenosine cation (I) which would undergo subsequent nucleophilic attack by the sulfur atom of methionine[3].



(I)

Although this sequence is only one of a number of possible alternatives, it was of interest to attempt the synthesis of the *cyclo* compound as a means of exploring further the mechanism of the enzymic reaction.

Although 3,5′-*cyclo*adenosine could be prepared from 2′,3′-*iso*propylidene-3,5′-*cyclo*adenosine[5] by acid hydrolysis, the conditions required for the removal of the protecting group (0.1 *M* HCl at 100° for 45 min) led to a complex mixture of products. A more satisfactory synthesis involved the removal of the *iso*propylidene group from 2′,3′-*iso*propylidene-5′-*p*-toluenesulfonyl adenosine[5,6] by treatment with formic acid saturated with HCl at ice-bath temperature for 30 min and subsequent cyclisation in boiling water. The ionic compound was then isolated as the reineckate, converted to the chloride salt, and repeatedly crystallised from aq. alcohol. 3,5′-*cyclo*Adenosine chloride (Found: C, 41.69; H, 4.37; Cl, 12.32; N, 24.77; calcd. for $C_{10}H_{12}O_3N_5Cl$: C, 41.59; H, 4.15; Cl, 12.31; N, 24.25) absorbed u.v. light maximally at 272 mμ, moved rapidly towards the cathode on paper electrophoresis and was susceptible to periodate oxidation. It decomposed, without melting, above 230°.

To test the suggestion that 3,5′-*cyclo*adenosine cation might be an intermediate in the enzymic activation of methionine, this compound has been tested as a precursor of S-adenosylmethionine using the methionine-activating enzymes of both liver and yeast.

In a preliminary experiment yeast enzyme was incubated in reaction mixtures containing [2-^{14}C]methionine, K^+, Mg^{++} and buffer, but with 3,5′-*cyclo*adenosine chloride (3.1 or 12.6 μmoles/ml) in lieu of ATP. After incubation, carrier S-adenosylmethionine was added and reisolated chromatographically[7]. In neither case was there an increase in the radioactivity of the column fraction containing S-adenosylmethionine over that found in the control from which methionine was omitted. From a parallel experiment using ATP (24.4 m*M*), it was calculated that formation of S-adenosylmethionine from the *cyclo*nucleoside at 10% of the rate found with ATP could have been readily detected. Similar results were obtained in a corresponding experiment using the methionine-activating enzyme of rabbit liver.

Although 3,5′-*cyclo*adenosine itself did not appear to be an intermediate in the activation reaction a second type of experiment was carried out to investigate the possible formation of an intermediate which could break down to the *cyclo*nucleoside

or to certain other compounds. In this experiment [8-^{14}C]adenosine triphosphate was used as substrate. Non-radioactive pools of 3,5′-*cyclo*adenosine, adenosine, and ADP were included in the reaction mixture and at the end of the incubation with enzyme were reisolated and assayed for radioactivity. To increase the chances of promoting a

TABLE I

RADIOACTIVITY OF NUCLEOSIDES ISOLATED AFTER INCUBATION OF [8-^{14}C]ATP WITH METHIONINE-ACTIVATING ENZYME OF YEAST

All vessels contained (in μmoles): either tris(hydroxymethyl)aminomethane buffer, pH 7.5, 50, or imidazole buffer, pH 5.6, 50 and sodium succinate, pH 5.6, 25. The complete reaction mixtures contained: KCl, 100; $MgCl_2$, 100; reduced glutathione, pH 6.7, 8; L-methionine, 22.5; adenosine, 2.65; 3,5′-*cyclo*adenosine (chloride salt), 2.6; ADP, pH 5.6 or 7.5, 2.4; [8-^{14}C]ATP, pH 6.7, 0.82 (containing $126 \cdot 10^3$ counts/min); yeast inorganic pyrophosphatase, alcohol fraction of HEPPEL[8] sufficient to hydrolyze 40 μmoles pyrophosphate under these conditions. Yeast methionine-activating enzyme, 5.9 units of an $(NH_4)_2SO_4$ A fraction[4]. Omissions were as indicated. Pyrophosphate, where present, 1 μmole (Na salt). The final vol. was 0.78 ml. The reaction was initiated by the addition of enzyme; the incubation was continued for 30 min at 37° and 1.2 ml 6 % trichloroacetic acid was added to stop the reaction. Adenosine 5′-phosphate (2.5 μmoles), ATP (6.8 μmoles), and S-adenosylmethionine (2 μmoles) were added as carriers. The mixture was centrifuged to remove the precipitated protein and the supernatant fluid extracted 3 times with several volumes of ethyl ether. The aqueous phase was neutralized and fractioned by the use of a Dowex-1-Cl column[9]. The material which was washed through the column by 10 vol. water (adenosine, 3,5′-*cyclo*adenosine, and S-adenosylmethionine) was further fractionated by passage over a column of IRC-50 (XE-64) (6 cm × 1 cm), buffered at pH 6.9. Adenosine was eluted[7] with 10 ml 0.01 *M* KPO_4, pH 6.9. Most of the 3,5′-*cyclo*nucleoside was eluted with 25 ml 0.25 *N* acetic acid. Elution was continued with this eluting fluid for an additional 25 ml, but this fraction, containing a few per cent of the 3,5′-*cyclo*adenosine and a small amount of the S-adenosylmethionine was discarded. S-Adenosylmethionine was then eluted with 4.0 *N* acetic acid. ADP, adenosine and 3,5′-*cyclo*adenosine were concentrated by adsorption on Norite A and elution with a small volume of 50% aq. ethanol containing 0.3 ml conc. NH_4OH in each 100 ml[10]. The charcoal was removed by filtration and aliquots of the filtrate were used to determine u.v. absorption and radioactivity. Aliquots of the S-adenosylmethionine and ATP fractions were counted without prior concentration. A thin-window, gas flow counter was used. All samples were corrected for self-absorption and for background. The specific activities were determined on the basis of the radioactivity and of the u.v. absorption (ε = 15, 400 at 259 mμ for the nucleotides, adenosine and S-adenosylmethionine; ε = 12,400 at 272 mμ for 3,5′-*cyclo*adenosine). The total radioactivity present in 3,5′-*cyclo*adenosine and adenosine could then be calculated since the net amount of these compounds did not change during the incubation. The total S-adenosylmethionine was assumed to be equal to the amount of carrier added after the incubation (2 μmoles) plus the amount estimated to have been synthesized during the reaction (a maximum of 0.8 μmole). This assumption introduces an uncertainty of no more than 20% in the amount of radioactivity in the S-adenosylmethionine. Calculated in this way, the total recovery of radioactivity ranged from 60–80%.

Omission	*pH*	*Total radioactivity (counts/min)*		
		3,5′-cyclo-adenosine	*Adenosine*	*S-adenosyl-methionine*
Enzyme	7.5	0	80	0
K^+	7.5	50	200	17,000
Methionine	7.5	10	70	0
Pyrophosphatase (pyrophosphate added)	7.5	50	330	52,000
None	7.5	10	390	84,000
Enzyme	5.6	0	110	0
K^+	5.6	0	110	200
Methionine	5.6	20	120	0
Pyrophosphatase (pyrophosphate added)	5.6	20	160	16,000
None	5.6	20	220	44,000

partial reaction and thus accumulating an intermediate, separate incubations were carried out in which either potassium or methionine was omitted from the complete reaction mixture or inorganic pyrophosphate was added in the absence of pyrophosphatase. A control vessel lacked methionine-activating enzyme. In addition, the entire set of incubations was duplicated at pH 5.6. This pH, although below the optimal pH for the enzyme, might be expected to cause cleavage of an acid-labile P–N bond and thus convert at least in part any hypothetical N-phosphorylated intermediate to a compound which would be trapped in one of the pools present.

The results of this experiment are shown in Table I. It may be seen that less than 0.5 % of the total radioactivity appeared in the 3,5'-*cyclo*adenosine or in the adenosine under any of the sets of conditions. These results appear to rule out the participation of either of these compounds as an intermediate and to make such a role for any N-phosphorylated derivative rather unlikely.

The results obtained with the reisolated ADP are less clear due to the possibility that traces of interfering enzymes were present. However the absence of isotopic dilution by the pool of ADP rules out the participation of free ADP as an intermediate in the conversion of ATP to S-adenosylmethionine, thus affording direct confirmation of a similar conclusion reached earlier in studies of the methionine-activating enzyme of rabbit liver[3].

As noted above, intermediate formation of free *cyclo*nucleoside cation was only one of a number of possible alternative reaction mechanisms leading to the formation of S-adenosylmethionine. Other possibilities still under investigation would require migration of the terminal phosphate of ATP to form a modified adenosine triphosphate. Alternatively, if the reaction proceeds via any intermediate, the latter may be visualized as enzyme bound.

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Laboratory of Cellular Pharmacology, S. H. MUDD
National Institute of Mental Health, G. A. JAMIESON
United States Public Health Service, Bethesda, Md. (U.S.A.) G. L. CANTONI

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