Enzymic Transfer of the Ribosyl Group from Inosine to Adenine*

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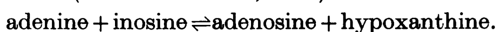
The formation of adenosine from adenine and inosine was first described by Stephenson & Trim (1938). Washed cell suspensions of *Escherichia coli* were used, and the formation of ammonia from adenine was stimulated by catalytic amounts of inosine. Stephenson & Trim cautiously postulated a transamination from adenine to inosine as a possible mechanism for the reaction. MacNutt (1952), working with a dialysed extract from *Lactobacillus helveticus*, demonstrated the transfer of the deoxyribosyl group from one purine or pyrimidine nucleoside to another purine or pyrimidine base. Orthophosphate was not required, nor was deoxyribose 1-phosphate involved in the reaction. The mechanism postulated was a direct transfer with the enzyme named trans-*N*-glycosidase. In the case of a transfer between hypoxanthine deoxyriboside and adenine, the same results would be obtained from transamination as from trans-*N*-glycosidation. The trans-*N*-glycosidase action was confirmed by experiments with radioactive adenine (Kalckar, MacNutt & Hoff-Jørgensen, 1952). Transamination was shown to play no part in the reaction.

Hoffmann (1952) described similar transfer reactions catalysed by extracts of *E. coli*. In these experiments the trans-*N*-glycosidase action occurred only between purines and purine nucleosides, or pyrimidines and pyrimidine nucleosides. Deoxyribose 1-phosphate was a necessary intermediate in a transfer from purine nucleoside to pyrimidine base, or from pyrimidine nucleoside to purine base.

It has recently been shown that cell-free extracts from *E. coli* catalyse the formation of adenosine and hypoxanthine from adenine and inosine (Ott & Werkman, 1954, 1955). The enzyme preparations contain nucleoside phosphorylase active on adenosine and inosine. Coupled nucleoside phosphorylase reactions were postulated as a mechanism for the reaction (Ott & Werkman, 1957)



$$\text{adenine} + \text{inosine} \rightleftharpoons \text{adenosine} + \text{hypoxanthine}.$$

The catalysis of the reaction by cell-free extracts in the absence of added orthophosphate indicates that trans-*N*-ribosidation or transamination plays a role in the mechanism of the reaction (Ott & Werkman, 1957).

The purpose of the present paper is to establish the presence or absence of transamination as a reaction mechanism in the formation of adenosine and hypoxanthine from adenine and inosine.

METHODS

Methods for the preparation of cell-free extracts have been previously described (Ott & Werkman, 1954). These extracts may be stored at −10° for at least a year with no appreciable decrease in activity.

All chemicals were commercial preparations. Adenine labelled with ^{14}C in C-8 (1·09 μc/mg.) was obtained from Schwarz Laboratories Inc., Mount Vernon, New York. Chromatographically the radioactive adenine was identical with non-radioactive samples of adenine. Direct counting of the spots from chromatograms showed that all of the radioactivity placed at the origin moved to the adenine location. Radioautography of the compound showed only one active spot that corresponded to adenine. Absorption in ultraviolet light corresponded to the adenine-absorption curve. The purity of all other purine and nucleoside preparations was verified by spectrophotometry and paper chromatography.

The reaction was conducted in 10 ml. Erlenmeyer flasks at 37° in a Dubnoff metabolic shaking incubator. Sodium pyrophosphate buffer, pH 6·5 (90 μmoles), [8-^{14}C]adenine (1·4 μmoles) and inosine (1·5 μmoles) or adenosine (1·8 μmoles), in a total volume of 0·8 ml., were incubated for 30 min. The reaction was initiated by the addition of 0·1 ml. of cell-free extract diluted with pyrophosphate buffer to contain 0·8 mg. of protein. The reaction was stopped by the addition of 0·1 ml. of 1·5 N-H_2SO_4.

Quantitative determination of the products was made on portions of the reaction mixture after removal of the protein precipitate by centrifuging. The products were separated by paper chromatography on Whatman no. 1 paper. Portions of the supernatant fluid were placed at the origin and neutralized on the paper with NH_3 soln. Development was carried out at 7° by the ascending method in water adjusted to pH 10 with NH_3 soln. (Levenbook, 1955). After drying at 100° for 10 min., the ultraviolet-light-absorbing spots were located with a Model SL Mineralight equipped with a 2537 Å filter. The purines and nucleosides were quantitatively determined by ultraviolet spectrophotometry of the eluted spots according to Vischer & Chargaff (1948). Elution was carried out overnight in N-HCl at room temperature. A Beckman DU spectrophotometer with 1·0 cm. cells was used for all determinations. To compensate for possible ultraviolet-light-absorbing materials in the filter paper, the

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concentration was determined from the density at the absorption maximum less the density at 290 mμ., as recommended by Vischer & Chargaff (1948).

Determinations of radioactivity were made on the compounds separated by paper chromatography. The spots that were visible under ultraviolet light were cut out and counted with a 1·4 mg./cm.^{2} end-window Geiger–Müller tube. All counts were made on paper.

Radioautography was carried out by exposing the developed chromatogram to Kodak No-Screen X-ray Safety Film for 3 days. The exposed film was developed according to the recommendations of the manufacturer.

Protein content of the extracts was determined by the biuret method of Weichselbaum (1946).

EXPERIMENTAL AND RESULTS

The formation of adenosine and hypoxanthine from adenine and inosine was carried out for 30 min. with [8-^{14}C]adenine as one substrate. The analyses of the reaction mixtures before and after incubation are shown in Table 1. Flasks 1–6 inclusive served as control flasks for substrate concentrations at zero time. Acid was added to these flasks before the enzyme preparation. No exchange of radioactivity occurred between adenine and inosine (flask 5) or between adenine and adenosine (flask 6) in the presence of enzyme inactivated by acid. The incubated flask containing adenine (flask 8) showed no decrease in concentration after incubation with the enzyme. Some decrease in concentration of inosine (flask 9) and adenosine (flask 10) occurred. This decrease was due to nucleoside phosphorylase activity of the cell-free extract with formation of the purine bases and ribose 1-phosphate (Ott & Werkman, 1957). The flask that contained [8-^{14}C]-adenine and inosine as substrate (flask 11) showed the formation of adenosine and hypoxanthine. Adenosine and adenine were the only radioactive compounds, with adenosine having essentially the same specific activity (13 800 counts/min./μmole) as the adenine (14 330 counts/min./μmole). The sum of the radioactivity found in the two compounds, 20 900 counts/min., corresponds satisfactorily with the activity in the corresponding flask at zero time, i.e. flask 5 with 20960 counts/min. This complete recovery of radioactivity indicates that there was no other radioactive compound formed from adenine. A comparison of flask 11 with flask 5 shows that there is essentially a 1:1 ratio of reactants disappearing to products formed. This stoicheiometry agrees with the results obtained when the reaction was carried out with non-radioactive compounds (Ott & Werkman, 1957).

An exchange of radioactivity occurs between adenine and adenosine in the presence of enzyme (flask 12). Essentially no change in concentration occurred during the incubation. Radioactive equilibrium was not reached in 30 min. At equilibrium the specific activity of each should be one-half of the specific activity of the original adenine or approximately 7000 counts/min./μmole.

Table 1. *Formation of radioactive adenosine from* [8-^{14}C]*adenine and inosine*

Indicated substrates were added to a test system which contained 0·1 ml. of cell-free extract (0·8 mg. of protein) and 90 μmoles of pyrophosphate buffer (pH 6·5). Total volume, 0·9 ml.; incubated for 30 min. at 37°. Protein was precipitated with 0·1 ml. of 1·5 N-H_2SO_4.

Flask no.	Substrate	Time of reaction (min.)	Compound determined	Radioactivity (counts/min.)	Quantity found (μmoles)	Change in quantity (μmole)	Specific activity (counts/min./μmole)
1	None	0	None	—	—	—	—
2	Adenine	0	Adenine	20 500	1·4	—	14 500
3	Inosine	0	Inosine	—	1·5	—	—
4	Adenosine	0	Adenosine	—	1·8	—	—
5	Adenine and inosine	0	Adenine	20 960	1·4	—	14 300
			Inosine	—	1·5	—	—
6	Adenine and adenosine	0	Adenine	19 700	1·4	—	14 000
			Adenosine	—	1·8	—	—
7	None	30	None	—	—	—	—
8	Adenine	30	Adenine	19 700	1·4	0·0	14 200
9	Inosine	30	Inosine	—	1·3	−0·2	—
10	Adenosine	30	Adenosine	—	1·6	−0·2	—
11	Adenine and inosine	30	Adenine	11 780	0·8	−0·6	14 350
			Adenosine	9 120	0·7	+0·7	13 800
			Hypoxanthine	0	0·6	+0·6	—
			Inosine	0	0·8	−0·7	—
12	Adenine and adenosine	30	Adenine	11 240	1·5	+0·1	7 750
			Adenosine	8 980	1·7	−0·1	5 210

The formation of radioactive adenosine from [8-^{14}C]adenine and inosine is shown by the radioautograph reproduced in Fig. 1. With adenine alone, no other radioactive spot was detectable. In the presence of inosine and enzyme, [8-^{14}C]adenine formed radioactive adenosine. The spots corresponding to hypoxanthine and inosine were devoid of activity. The exchange of radioactivity between adenine and adenosine is also shown on the radioautograph.

DISCUSSION

The formation of radioactive adenosine from [8-^{14}C]adenine and inosine establishes the mechanism of the reaction as a transfer of the ribosyl group. If the amino group had been transferred from adenine to inosine, the hypoxanthine formed would have been radioactive. Only by a transfer of the ribosyl group would radioactivity be found in adenosine. The absence of radioactivity in the hypoxanthine formed during the reaction rules out a transamination reaction. The essentially undiluted specific activity of the adenosine indicates that it was formed only from adenine and that there were few, if any, side reactions. The 1:1 ratio of reactants disappearing to products formed further indicates the absence of side reactions. Although the cell-free extract shows nucleoside phosphorylase activity on inosine and adenosine, the rates of the two reactions are such that there is no net synthesis of ribose 1-phosphate when both adenine and inosine or adenosine and hypoxanthine are incubated together (Ott & Werkman, 1957).

The transfer of the ribosyl group was demonstrated from inosine to adenine and from adenosine to adenine. Since the reaction is reversible (Ott & Werkman, 1957), it is likely that the ribosyl group can be transferred from adenosine to hypoxanthine.

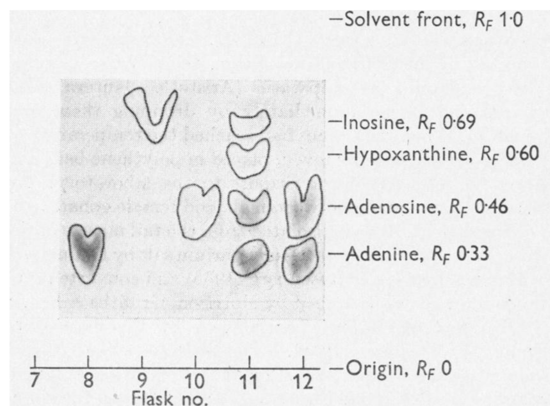

Fig. 1. Radioautograph of the formation of adenosine and hypoxanthine from [8-^{14}C]adenine and inosine. Test system and conditions were the same as in Table 1. Spots shown in outline were those visible under ultraviolet light. Film was exposed to the chromatogram for 3 days.

The ribosyl transfer is similar to the deoxyribosyl transfer in *L. helveticus* with the analogous deoxyribose compounds as described by Kalckar *et al.* (1952). In both cases transamination has been eliminated as a reaction mechanism. The mechanism with the deoxyribosyl compounds and the *L. helveticus* preparations was shown to be a direct transfer with no involvement of deoxyribose 1-phosphate (MacNutt, 1952). The nucleoside phosphorylase activity of the *E. coli* extracts and the reactions of the purine bases with ribose 1-phosphate to form the nucleosides (Ott & Werkman, 1957) indicate that the reaction mechanism in *E. coli* involves a ribosyl transfer with ribose 1-phosphate as the intermediate. The possibility of a direct transfer of the ribosyl group with *E. coli* extracts has not been eliminated. The data obtained (Ott & Werkman, 1957) indicate that the reaction is the result of coupled nucleoside phosphorylase reactions. Enzyme preparations from *E. coli* that catalyse the reaction in the complete absence of orthophosphate or that are devoid of nucleoside phosphorylase activity have not been obtained. The amount of orthophosphate present in the extract was 0·16 μmole (cf. Ott & Werkman, 1957, for discussion).

SUMMARY

1. The formation of radioactive adenosine from [8-^{14}C]adenine and inosine catalysed by cell-free extracts from *Escherichia coli* establishes the transfer of the ribosyl group as a mechanism of the reaction. Hypoxanthine formed during the reaction was devoid of radioactivity, a result that eliminates transamination as a mechanism.

2. The involvement of ribose 1-phosphate as an intermediate in the reaction and the relationship of the reactions to the deoxyribosyl transfer in *Lactobacillus helveticus* preparations is discussed.

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