EVIDENCE FOR MORE THAN ONE PATHWAY IN THE FORMATION OF PURINE DEOXYRIBONUCLEOTIDES

A.FOUNG and A.W.NORMAN

Department of Biochemistry, University of California, Riverside, California 92502

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The biosynthesis of purine deoxyribonucleotides was studied in the context of general purine nucleotide metabolism in the chick with the aid of radioactive nucleic acid precursors. Our results showed that in chick liver and intestine, the nucleoside phosphate reductase system so firmly established in *E. coli* [1] and *L. leichmanni* [2] is not exclusively responsible for the biosynthesis of purine deoxyribonucleotides.

1. Introduction

The biosynthesis of purine deoxyribonucleotides has not been studied as extensively as that of the pyrimidine deoxyribonucleotides. It was found in chicken embryo [3], *E. coli* [1,4], and Novikoff tumor cells [5] that the cytidine phosphate reductase could, under some circumstances, also catalyze the reduction of purine ribonucleotides to deoxyribonucleotides. However, there are no data available from *in vivo* experiments which indicate that the direct reduction of purine ribonucleotides could exclusively account for the biosynthesis of all the purine-bound deoxyribose.

In this communication, we investigated the problem of the origin of purine-bound deoxyribose in chick liver and intestinal mucosa. The problem was approached with the assumption that ribonucleotides are the direct precursors of deoxyribonucleotides. Thus, if a uniformly labeled nucleoside is supplied to a chick, the ratio of the specific activity of the carbohydrate portion to the specific activity of a base portion should be the same for ribonucleosides and the corresponding deoxyribonucleosides isolated from RNA and DNA. The results obtained showed that this assumption was not valid.

2. Experimental procedure

In each experiment twelve chicks were used. Ten chicks received equal amounts of ^{14}C-nucleoside and were paired off as five replicates during the isolation of DNA and RNA. Two chicks were injected with 0.9% NaCl and served as blanks.

^{14}C-adenosine, ^{14}C-guanosine or ^{14}C-cytidine were used as nucleic acid precursors in separate experiments. 0.25 ml of the carrier free radioactive nucleosides (1 μc of ^{14}C-adenosine, 0.5 μc of ^{14}C-guanosine and 0.5 μc of ^{14}C-cytidine per chick) were injected intracardially. The chicks were sacrificed 48 hours after injection. DNA and RNA were isolated from liver and intestine by the NaCl method [6]. After hydrolyzing the nucleic acids to the nucleoside stage, the nucleosides were separated by column chromatography [7]. Specific activity (dpm/μmole) was determined for each nucleoside.

The purine ribonucleosides and deoxyribonucleosides were then hydrolyzed without damage to either the carbohydrate portion or purine bases according to the method described by Roll, Weinfeld, Carroll and Brown [8]. The radioactivities in the two portions were measured in a Nuclear-Chicago liquid scintillation counter.

The pyrimidine nucleosides were hydrolyzed with 97% formic acid in a bomb tube [9]. Cytosine was separated from the other decomposition products by adsorption onto acid-washed charcoal. It was then eluted, concentrated and its specific activity determined. Specific activity of deoxyribose or ribose could be calculated from the difference of the known specific activities of cytidine and cytosine.

3. Results and discussion

The forty-eight hour interval between injection of radioactive precursor and sacrifice of the chicks was to insure maximal incorporation of the nucleosides. However, only 0.3-3.0% of the radioactivity in the precursors was incorporated into nucleic acids. When ^{14}C-adenosine or ^{14}C-guanosine was the precursor, 70-90% of the incorporated radioactivity was distributed between adenosine and guanosine, whereas 0-4% of the incorporated radioactivity was found in the purine nucleosides with ^{14}C-cytidine as the precursor.

After DNA and RNA were hydrolyzed to their nucleosides, ratios of radioactivity in the carbohydrate portion to the radioactivity in the base portion were obtained for both purine nucleosides when ^{14}C-adenosine or ^{14}C-guanosine was the precursor. Ratios were obtained for pyrimidine nucleosides when ^{14}C-cytidine was the precursor. The values of the ratios are summarized in table 1.

The ratios found for purine ribonucleosides isolated from liver and intestine nucleic acids differ significantly from the ratios of the precursors. This indicated that the glycosidic bond in the precursor was broken after it entered the organism. The specific activity in either components was expected to decrease by a factor depending on the endogenous pool size of

Table 1

Ratios of radioactivity in the carbohydrate portion to the base portion of nucleosides of chick liver and intestine DNA and RNA after injection of radioactive precursors.

	Precursor nucleoside					
	^{14}C-adenosine [3]		^{14}C-guanosine [4]		^{14}C-cytidine [5]	
Ratio of precursor nucleoside	1.30		1.12		1.45	
Nucleosides isolated from Nucleic Acids	R [1]	dR [2]	R	dR	R	dR
Liver						
Cytidine					-- [6]	0.69
Adenosine	0.76	0.39	5.77	2.39		
Uridine or Thymidine					--	1.20
Guanosine	0.73	0.24	1.79	0.68		
Intestine						
Cytidine					0.71	0.50
Adenosine	0.18	0.168	1.26	1.11		
Uridine or Thymidine					--	1.34
Guanosine	0.18	0.042	0.64	0.09		

[1] R = ribosides.
[2] dR = deoxyribosides.
[3] Ratios are average values of twelve replicates.
[4] Ratios are average values of six replicates.
[5] Ratios are average values of four replicates.
[6] Hydrolysis failed in this case.

the individual component and their turnover rates. Upon their recombination, we would obtain a nucleoside showing a different $\frac{^{14}C\text{-ribose}}{^{14}C\text{-purine}}$ ratio. It is interesting to note that, in general, the ratios decrease when ^{14}C-adenosine was the precursor and increase when ^{14}C-guanosine was the precursor. This would imply different fates for injected adenosine and guanosine.

In liver, the purine deoxyribosides have lower ratios than corresponding ribosides. It is clear then, that direct reduction of ribonucleotides to deoxyribonucleotides is not the only reaction by which purine bound deoxyribose is formed, if such a pathway does exist.

In intestine, guanosine and deoxyguanosine showed different ratios, but similar ratios were found for adenosine and deoxyadenosine. It is possible that different biosynthetic pathways exist in different organs and for different, though closely related, substrates. Here it appears that the direct reduction contributes significantly in the formation of deoxyadenosine.

The experiment with ^{14}C-cytidine as the nucleic acid precursor was done with the purpose of confirming Reichard's work in regenerating rat liver [10] where he obtained the same $\frac{^{14}C\text{-sugar}}{^{14}C\text{-base}}$ ratios for all pyrimidine nucleosides as for the precursor. This situation arose only because exogenous cytidine was not metabolized to any great extent before being incorporated into the nucleic acids [11]. Our results showed that such is not the case in chick. Reichard's result is partially confirmed in chick intestine. It is difficult to explain the higher ratio found in thymidine than in deoxycytidine from our present concepts of thymidine biosynthesis.

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