A Study of the Biosynthesis of Deoxyribose in Wheat Embryos*

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ABSTRACT: Glucose labeled in various carbons with ^{14}C was fed to wheat embryos for four different time periods. Deoxyribose was isolated and sequentially degraded to determine the position of labeling. When glucose-6-^{14}C was supplied, radioactivity first appeared in C_5 while longer feeding periods resulted in some label incorporation in C_3 followed by random labeling of C_1, C_2, and C_4. Embryo feeding with glucose-2-^{14}C resulted in radioactivity appearing in C_1, C_2, and C_4 with randomization at long feeding periods. Feeding glucose-1-^{14}C resulted in label appearing first in C_1 and C_5 followed by C_3 and random labeling of C_2 and C_4.

On the basis of these results, it is suggested that a combination of the oxidative and nonoxidative pathways is involved.

Since nucleic acids have been found to have such important roles in living organisms, the biosynthesis of nucleic acids and their sugar moieties have become a common subject of research in recent years. Indeed the presence of 2-deoxy-D-ribose is one of the distinguishing features of DNA, yet the mechanism of biosynthesis of this compound is relatively obscure.

Racker (1952) first postulated a possible metabolic pathway for deoxyribose on the basis of studies with an *in vitro* system. Numerous studies of the biosynthesis of deoxyribose in microorganisms and animal tissues followed. This research has been reviewed by Sable (1966). In general, when glucose-^{14}C was supplied to bacteria or animal tissue, the pattern of labeling in ribose and deoxyribose was similar, suggesting that deoxyribose is produced directly from ribose or that these two pentoses have a common precursor. Biosynthesis of deoxyribose is thought to occur *via* the oxidative pathway (glucose conversion into pentose through the glucose 6-phosphate dehydrogenase reaction) or *via* the nonoxidative pathway (glucose conversion into triose in reactions of glycolysis and thence to pentose by transaldolase and transketolase reactions).

This study was undertaken to obtain information on the biosynthesis of deoxyribose in higher plants where there is at present very limited or no information available.

Materials and Methods

Treatment of Embryos. Embryos were isolated in mass by the method of Johnston and Stern (1957), from seed of common bread wheat (*Triticum aestivum*). Samples of embryos from approximately 160 g of wheat were incubated for 6-, 18-, and 48-hr periods in glucose-1-^{14}C, glucose-2-^{14}C, or glucose-6-^{14}C. Each sample contained approximately 3.5 μCi of labeled glucose in 30 ml of water.

Extraction of DNA. DNA was extracted by a modification of the procedure of Colter *et al.* (1962). Embryos were ground in phenol buffer solution in a glass homogenizer. The solution contained 1.5 ml of 10% aqueous sodium deoxycholate, 28.5 ml of a solution 1 M in sodium chloride and 0.02 M in potassium phosphate (pH 7.4), and 30 ml of water-saturated phenol. The suspension was made up to 60 ml with phenol buffer, then centrifuged for 10 min at 17,300*g*. The upper aqueous layer containing the DNA was extracted twice with equal volumes of phenol buffer. Traces of phenol were removed from the aqueous DNA solution by ether extraction. An equal volume of 95% alcohol was added and the DNA, which appeared as long fibers, was collected on a glass rod.

Isolation of Deoxyribose. The DNA was dissolved in 0.02 M magnesium sulfate, by adding 1 N ammonium hydroxide to pH 7.6. The solution was treated with deoxyribonuclease (Nutritional Biochemical Corp.) at 37° for 6 hr. The medium was then adjusted to pH 9 with glycine buffer and incubated with snake venom enzyme (Sigma Chemical Co.). The mixture was centrifuged at 4340*g* and the supernatant containing deoxyribonucleosides was removed. The solution of deoxyribonucleosides was treated with a few drops of bromine for 15 min then sodium hydroxide was added to pH 11. After 1 hr, pH was adjusted to 1.5 with 1 N HCl and hydrolysis was carried out at 100° for 15 min to free the deoxyribose from purine and pyrimidine bases. The hydrolysate was passed through Dowex 50-X8 (H^+) and Duolite A-4 (OH^-) resin and the effluent containing deoxyribose and other neutral sugars was collected. The sugar solution was concentrated by vacuum evaporation and strip was loaded on Whatman No. 1 paper. The chromatogram was developed with ethyl acetate–acetate–water (8:2:2, v/v) as a descending solvent for 16–20 hr. Sugars were located with *p*-anisidine reagent and the deoxyribose was eluted with distilled water.

Degradation of Deoxyribose. Deoxyribose was sequentially degraded by the method of Unrau and Canvin (1963). Labeled carbon dioxide was trapped in ethanolamine–methyl Cellosolve (1:2, v/v) and counted by liquid scintillation spectrometry. A background count was made on each vial containing the scintillator solution prior to adding the sample. Background and sample counting time was 100 min.

The complete degradation of deoxyribose is illustrated below. Uniformly ^{14}C-labeled deoxyribose was also degraded in

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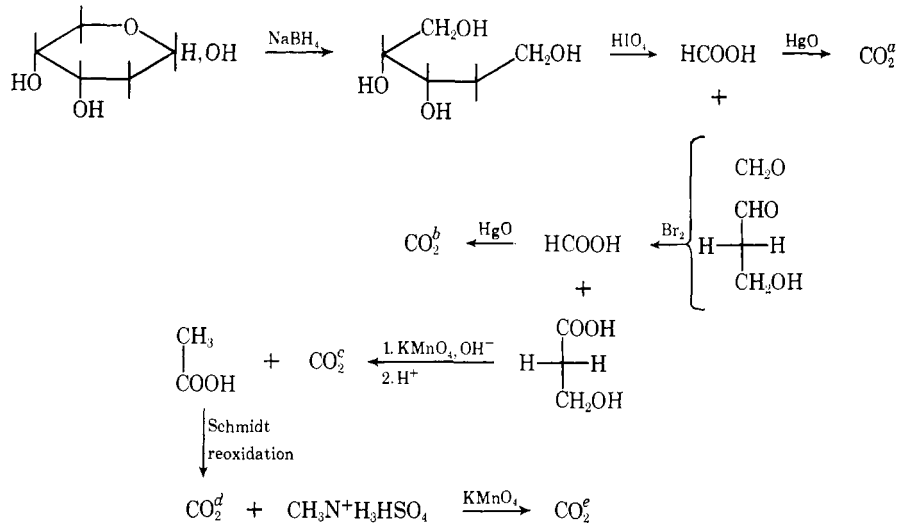
SCHEME I: Degradation Sequence of 2-Deoxy-D-ribose



[a] CO_2 from C_4 of deoxyribose. [b] CO_2 from C_5 of deoxyribose. [c] CO_2 from C_3 of deoxyribose. [d] CO_2 from C_1 of deoxyribose. [e] CO_2 from C_2 of deoxyribose.

the illustrated fashion for a further check on yield on each step.

Results and Discussion

Studies with microorganisms and animals have shown that there exist two principal pathways for the biosynthesis of pentose from hexose. The most direct route for the synthesis of pentose from hexose is the oxidative pathway which involves the decarboxylation of 6-phosphogluconate to ribulose 5-phosphate. By this pathway, C_2 through C_6, respectively, of glucose furnishes C_1 through C_5, respectively, of ribose, while C_1 of glucose is lost as carbon dioxide. If recycling occurred, redistribution of the first three carbon atoms from glucose would take place during the second, third, and subsequent cycles.

The second route is the nonoxidative synthesis of pentose from hexose which involves the reactions catalyzed by transaldolase and transketolase. These reactions require stoichiometric quantities of glyceraldehyde 3-phosphate, which could arise in different manners. The metabolic sequence giving rise to glyceraldehyde will determine, to a great extent, the possible disposition of carbon from glucose to deoxyribose. If glyceraldehyde phosphate is produced by the pentose phosphate cycle, then the three carbons of this triose are derived solely from C_4, C_5, and C_6 of glucose. On the other hand, if glucose is also metabolized by the Embden–Meyerhof–Parnas (EMP) pathway, glyceraldehyde 3-phosphate could arise from C_1, C_2, and C_3 or C_4, C_5, and C_6 of glucose. The following carbon atoms of glucose become equivalent in the triose as a result of the triose phosphate isomerase reaction: C_1 and C_6, C_2 and C_5, and C_3 and C_4.

If the Entner–Doudoroff pathway takes part in the metabolism, then glyceraldehyde 3-phosphate would be derived from C_1, C_2, and C_3 of glucose, and C_1 of glyceraldehyde 3-phosphate would correspond to C_1 of glucose. If glyceraldehyde 3-phosphate originates from the EMP or Entner–Doudoroff pathway and is utilized in the production of pentose *via* transaldolase and transketolase reactions, then the carbons of glucose are distributed as follows in pentose: $C_1 = C_1, C_5$; $C_2 = C_2, C_4$, and C_3; $C_4 = C_3$; $C_6 = C_5$.

An alternate pathway is the condensation of acetaldehyde and triose phosphate. The low affinity of deoxyribose phosphate aldolase for acetaldehyde suggests that this enzyme may be important in the degradation but not in the synthesis of deoxyribose. Still another pathway is from glucose *via* glucuronic acid and xylulose with loss of C_6 of glucose. This cycle was first suggested in 1956 (Hollmann and Touster, 1956) and some experiments (Moscatelli and Larner, 1959; Neufeld *et al.*, 1958; Hassid *et al.*, 1959; Utter, 1958; Hollmann, 1964) support the theory that C_6 of glucose is converted into carbon dioxide. These experiments do not, however, suggest that this glucuronate–xylulose cycle has any significant quantitative role in carbohydrate metabolism.

Glucose-6-^{14}C Feeding Experiments. Of the four possible metabolic pathways of deoxyribose biosynthesis previously discussed, the condensation of acetaldehyde and glyceraldehyde 3-phosphate by the enzyme deoxyribose phosphate aldolase has received little support in that the enzyme affinity for acetaldehyde is too low to form any significant amount of product *in vivo* (Wright *et al.*, 1961; Fossitt and Bernstein, 1963; Ghosh and Bernstein, 1963; Hammersten *et al.*, 1950). The glucuronic acid–xylulose cycle appears to be relatively unimportant for the biosynthesis of deoxyribose and ribose, at least in animals (Hiatt and Lareau, 1958). In this study only the oxidative pathway and nonoxidative pathway are considered since none of the other pathways are generally indicated by the results including those from the 48-hr feeding period (Tables I–IV).

Essentially all of the radioactivity derived from glucose-6-^{14}C was found in C_5 of deoxyribose in the 6- and 18-hr feeding experiments (Table II). The 48-hr feeding period resulted in some random labeling of C_1, C_2, C_3, and C_4. This can be explained by recycling particularly if both the oxidative and non-

TABLE I: Degradation of 2-Deoxy-D-ribose, 2-Deoxy-D-ribose-1-^{14}C, and 2-Deoxy-D-ribose-U-^{14}C.

	Barium Carbonate (mg)				
	C_1	C_2	C_3	C_4	C_5
Deoxyribose					
1 (40 mg)	35 (39)[a]	34 (39)	40 (39)	41 (39)	42 (39)
2 (30 mg)	24 (29)	25 (29)	30 (29)	32 (29)	32 (29)
3 (20 mg)	17 (19.5)	17 (19.5)	20 (19.5)	22 (19.5)	21 (19.5)
Deoxyribose-1-^{14}C					
1 (1650 dpm)	1520	25			
2 (3600 dpm)	3490	35			
Deoxyribose-U-^{14}C					
1 (1650 dpm)	305	300	340	315	335
2 (3600 dpm)	695	700	735	730	720

[a] Figures enclosed by parentheses represent experimentally expected yields.

oxidative scheme are invoked. Randomization due to bacterial fragmentation of the administered labeled deoxyribose was insignificant since examination of the embryo cultures at 48 hr showed only a minor bacterial count. Carbon-3 contained a minor amount of label at 18 hr and at 48 hr observably more label than C_1, C_2, and C_4. This could be due to a minor participation of the Entner–Doudoroff pathway. The appearance of label more or less exclusively in C_5 indicates that either one or both (oxidative, nonoxidative) pathways are involved.

Glucose-1-^{14}C Feeding Experiments. The relevant data of Table III show that approximately 64% of the radioactivity in deoxyribose was in C_5 and 36% in C_1 after 6 hr. After 18 hr, about 54% of the total label appeared in C_5, about 44% in C_1, and a minor amount appearing in C_3. At 48 hr, 46% of total label appeared in C_5, 40% in C_1, and 14% in C_2, C_3, and C_4. As suggested previously, some random labeling of C_2, C_3 and C_4 after the 48-hr feeding period may be explained by recycling involving both the oxidative and the nonoxidative scheme. In both pathways, glyceraldehyde 3-phosphate from the EMP pathway could contribute label to C_5; however, only the nonoxidative pathway would contribute label to C_1 and C_5. Both pathways may be involved although the nonoxidative pathway alone could account for the label incorporation from glucose-1-^{14}C.

Glucose-2-^{14}C Feeding Experiments. The data in Table IV show that at the 6-hr feeding period 25% of total activity is in C_4, 25% in C_2, and 50% in C_1 of deoxyribose. At 18 hr, 26% is in C_4, 29% in C_2, 37% in C_1, and about 9% in C_3 and C_5 combined.

If the nonoxidative and EMP pathways are involved, C_2 and C_4 would be expected to have high radioactivity. The high radioactivity of C_2 and C_4 (Table IV) is in agreement with this hypothesis, while the radioactivity of C_1 would be due to involvement of the oxidative pathway.

Previous research has, in general, indicated that both ribose and deoxyribose are synthesized *via* the same general pathways or from common precursors. In most cases, both ribose and deoxyribose bear a similar labeling pattern when derived from glucose-^{14}C although a notable exception to this was found by Horecker *et al.* (1958) and Laland and Kielland (1956) in their work with animal tissue. Biosynthesis of ribose appears to generally proceed *via* a combination of the oxidative and nonoxidative pathways (Sable, 1966) with a variation in the degree of participation dependent upon the organism studied.

TABLE II: Distribution of ^{14}C in Deoxyribose from Wheat Embryos Fed Glucose-6-^{14}C.

Feeding Period (hr)		dpm				
		C_1	C_2	C_3	C_4	C_5
6	1	Nil	Nil	Nil	Nil	55
	2	Nil	Nil	Nil	Nil	75
18	1	Nil	Nil	15	Nil	130
	2	Nil	Nil	10	Nil	165
48	1	45	40	65	35	290
	2	35	30	70	40	330

TABLE III: Distribution of ^{14}C in Deoxyribose from Wheat Embryos Fed Glucose-1-^{14}C.

Feeding Period (hr)		dpm				
		C_1	C_2	C_3	C_4	C_5
6	1	25	Nil	Nil	Nil	45
	2	45	Nil	Nil	Nil	65
18	1	80	Nil	Nil	Nil	95
	2	85	Nil	10	Nil	105
48	1	200	15	35	20	230
	2	215	20	40	30	240

TABLE IV: Distribution of ^{14}C in Deoxyribose from Wheat Embryos Fed Glucose-2-^{14}C.

Feeding Period (hr)		dpm				
		C_1	C_2	C_3	C_4	C_5
6	1	65	30	Nil	30	Nil
	2	75	40	Nil	35	Nil
18	1	150	120	20	110	15
	2	175	130	20	115	25
48	1	225	200	60	190	40
	2	290	240	70	230	80

The data presented indicate a definite participation of both the oxidative and nonoxidative pathways. In such a sequence of metabolic events, triose phosphate becomes a key compound, since it may be derived both from the EMP pathway and *via* the Entner–Doudoroff pathway. Whether or not deoxyribose arises from a direct reduction of C_2 of ribose is under investigation. Some preliminary studies with ribose-1-^{14}C have been carried out and indicate a direct reduction to 2-deoxyribose (A. M. Unrau, 1969, unpublished results). The results obtained bear a close similarity to the deoxyribose labeling pattern presented by previous workers (Horecker *et al.*, 1958; Laland and Kielland, 1956), and consequently indicates some similarity in the pathway between animals and higher plants.

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