HETEROGENEITY OF $^{32}PO_4^{3-}$ INCORPORATION IN αPO_4^{3-} OF FREE RAT LIVER NUCLEOTIDES

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Received 18 November 1968

The amount of acid-soluble triphosphates is different from one tissue to the other. The existence of two types of free nucleotide distribution was suggested: one an energetic type, rich in adenylic nucleotides found in muscle, brain, lens, the other a metabolic type, with a relatively high level of GTP and UTP, distribution found in liver [1,2]. Concerning CTP it was shown that in tumor cells the amount of this nucleotide is higher than in non-proliferating tissue [3] and that CTP decreases to reach a low level at the end of growth [4,5]. Thus it was suggested that CTP or a step of the CTP producing pathway, may be a limiting factor in growth [6,7].

The tissue level of free nucleotides represents an equilibrium between degradation and biosynthesis. The low tissue level does not mean necessarily that CTP biosynthesis is decreased in adult animals. In order to evaluate the level of CMP synthesis compared to that of the other free nucleotides, we have studied the incorporation of $^{32}PO_4^{3-}$ in αPO_4^{3-} of free nucleotides in normal adult rat liver after different labelling times. It was generally admitted that after a delay of two days ^{32}P labelled free nucleotides are in equilibrium and that the specific activity of the α phosphates of the different nucleotides have similar values. This paper reports that even after 4 days the specific activity of αPO_4^{3-} of CMP is still lower than that of the other nucleotides. This kind of data has also an interest in the evaluation of base ratios in labelled RNA using the ratio of radioactivity of the four nucleotides.

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Each experiment was carried out on two adult rats of Wistar strain weighing 200–250 g. The animals received intravenously $^{32}PO_4^{3-}$ 300 μCi for 100 g of body weight, 2, 6, 10, 24, 48 and 96 h before sacrifice. The extraction of the acid-soluble free nucleotides has been performed according to methods described elsewhere [8]. The total nucleotide extract has been concentrated by lyophilisation and then hydrolyzed with saturated barium hydroxyde as described by Kerr [9]. The resulting nucleoside monophosphates were submitted to purification on charcoal columns. After elution with alcohol–NH_3–water (50:10:40, v/v/v), the nucleotides were separated by chromatography on anion exchange resin column Dowex 1 × 8, carried out according to the technique of Cohn [10] and Hurlbert and coworkers [11]. The specific activity of the four nucleosides monophosphates was determined on the pooled fractions corresponding to each nucleotide by UV optical density measurement and by radioactivity determinations with a Tricarb liquid scintillation spectrometer. Our results are shown in fig. 1.

The data show great differences in specific activities of the αPO_4^{3-} of nucleotides. In 2 h duration label experiments the specific activity of AMP is at least three times higher than that of UMP, GMP and CMP (specific activity of AMP 29310, UMP 8850, GMP 7257, and CMP 5041; counts/min/μg nucleotidic P). Thus in short label experiments as used in messenger RNA studies, there is a great heterogeneity of the different free nucleotide specific activities. Therefore, when RNA base composition is measured after alkaline hydrolysis by radioactivity we have to assume that there is a random distribution of the labelled nucleotides in RNA preparation and that the transfer

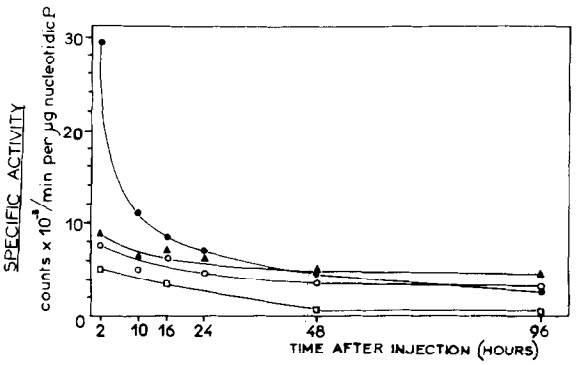

Fig. 1. Evolution of the specific activities of αPO_4^{3-} from free nucleotides in normal rat liver, after $^{32}PO_4^{3-}$ administration *in vivo*. □—□ CMP. ●—● AMP. ○—○ GMP. ▲—▲ UMP.

of the label from the 5′-phosphates on position C_3 on the near neighbour RNA nucleotides can lead to distribution of DNA-like RNA although CMP has a low specific activity in the free nucleotide pool. Even after a 10 h label the specific activity of AMP is still distinctly higher than those of UMP, GMP, and CMP. There is only a tendency to equilibrium after a long time of labelling, higher than 48 h. But even after a label of 96 h the values of specific activities of the four nucleoside monophosphates are not identical.

It is worth to note that the specific activity of CMP is always the lowest compared to that of three others independently of the label duration studied. Even after a long time of label (96 h) the specific activity of CMP (435 counts/min/µg nucleotidic P) is $\frac{1}{5}$ of that of AMP (2530 counts/min/µg nucleotidic P).

The data reported are in agreement with our previous statements, the very low quantity of free cytidylic nucleotides in normal liver compared to that in regenerating liver, in hepatoma [3] and the decrease of CTP and the end of course of rat brain growth [4]. Not only the amount of free CTP is the lowest among the four nucleotides in resting animal cells, but also its biosynthesis as it is shown here. These findings are in favour to the suggestion that free cytidylic nucleotides are a limiting factor in tissue growth in animal cells.

It is important to point out the difference in labelling of α-phosphate of the free nucleotides at least in liver several hours after $^{32}PO_4^{3-}$ administration. It should be taken in account in the evaluation of base ratio in RNA using radioactivity determination of the four nucleotides in studies of the regulation of RNA synthesis.

This work is a part of the "Doctorat ès-Sciences" thesis of M.E.Ittel and has been done with the technical assistance of Mr. P.Vaurs.

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