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Nucleotides as semiessential nutritional components

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Dietary nucleotides are required nutrients for some tissues under certain circumstances. A lack of dietary nucleotides negatively influences protein synthesis in both the liver and the small intestine of rats. Ribosome degradation has been observed as being among the mechanisms responsible for this effect. Dietary nucleotides can also modulate gene expression by interaction with specific transcription factors, in both the liver and the small intestine.

Dietary nucleotides: Protein synthesis: Gene regulation

Introduction

Nucleotides are normal components of the human diet and the body provides mechanisms for their absorption and incorporation into tissues (for a review see Sánchez-Pozo *et al.* 1998). These compounds are not considered to be essential nutrients because they can be synthesized endogenously. In fact, no particular disease has been related to a nucleotide deficiency. However, in certain circumstances, and for some tissues, a lack of dietary nucleotides may impair important functions, suggesting a key nutritional role.

Cellular proliferation, among other biological processes, requires significant amounts of nucleotides. Synthesis of nucleotides from amino acids and phosphoribosylpyrophosphate is an energy consuming process and therefore the utilization of exogenous nucleotides may be beneficial from a bioenergetic point of view. In the absence of exogenous nucleotides the *de novo* synthesis is thought to be activated (Yamaoka *et al.* 1997). Some tissues such as the lymphoid tissue (Perignon *et al.* 1987) or the intestine (Leleiko *et al.* 1983) have a low biosynthetic capacity, probably being dependent on an exogenous supply (Uauy, 1989; Van Buren & Rudolph, 1997).

Dietary nucleotides may also be conditionally essential nutrients in a variety of clinical situations and during development. Thus, it has been shown that they accelerate the recovery of the liver and small intestine after a variety of insults (Núñez *et al.* 1990; Bueno *et al.* 1994; Uauy *et al.* 1994; Jackson *et al.* 1997; Torres *et al.* 1997; Tsujinaka *et al.* 1997; Yamamoto *et al.* 1997). They also promote maturation in both the liver and the small intestine (Uauy, 1989; Carver, 1994; Ortega *et al.* 1995*a*). Maturation of the small intestine is particularly important in neonates because of their rapid growth, especially in low-birth-weight infants due to their intrauterine malnutrition. Among other actions, dietary nucleotides have a significant effect in immunity (Carver, 1994; Yamamoto *et al.* 1997; Carver, 1999; Rueda & Gil, 2000). Therefore, nucleotide supplementation of formulas for infant nutrition or for parenteral nutrition is considered beneficial.

The mechanisms by which dietary nucleotides exert their effects are not fully understood. However, there are data to suggest that they affect some biosynthetic processes such as the synthesis of proteins. Furthermore, there is evidence that they can modulate gene expression.

Dietary nucleotides and biosynthetic processes

When the rat diet does not contain nucleotides there is a transient decrease in the RNA content of the liver (López-Navarro *et al.* 1995). The lack of dietary nucleotides slightly affects the total concentration of soluble nucleotides, whereas the decrease in RNA is significant. We observed a reduction of the number of ribosomes associated with the endoplasmic reticulum as well as a reduction in the size of the nucleolus of the cells (López-Navarro *et al.* 1996*a*). Additional experiments showed that these changes are proportional to the nucleotide content of the diet. These findings indicate that ribosome formation is reduced and, what is more remarkable, pre-existing ribosomes are degraded in response to a lack of nucleotides in the diet. We think that these observations indicate a buffering role of RNA, which can protect the cell from nucleotide depletion while the biosynthesis responds to the lack of nucleotides from the diet. This buffering effect is conceivable, as there are no cellular stores of nucleotides. As a consequence, protein synthesis is decreased (López-Navarro *et al.* 1996*b*).

In the small intestine of rats, we have also found a

Abbreviations: CNT1, concentrative nucleoside transporter; IEC, intestinal epithelial cell; HPRT, hypoxanthine phosphoribosyl transferase; mRNA, messenger RNA; SPNT, sodium-dependent purine nucleoside transporter.

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decrease in RNA and soluble nucleotides, and consequently in protein synthesis when a nucleotide-free diet is fed. In addition, restriction of dietary nucleotides caused a decrease in DNA content (López-Navarro *et al.* 1996*b*). The changes were not transient as in the case of the liver and a degree of mucosal atrophy was observed morphologically and enzymatically (Ortega *et al.* 1995*a,b*).

These findings are consistent with the many studies in which it has been shown that tissue recovery or maturity are positively affected by dietary nucleotides. It is noteworthy that the contribution of dietary nucleotides to maintain nucleic acid levels is dependent on the tissue growth rate, affecting RNA in resting cells such the liver and also DNA in proliferating cells such the intestine.

In conditions of dietary nucleotide restriction, nucleotide diphosphates are significantly reduced in both the liver and the small intestine. These findings point to other relevant biosynthetic effects, as they are involved in many biosynthetic processes such as glycogen synthesis through uridine diphosphate derivatives, phospholipids through cytidine diphosphate derivatives and protein glycosylation. With regard to proteins, we believe that not only protein synthesis but also secretion of proteins may be influenced by dietary nucleotides. This is consistent with many studies showing a reduction of secreted proteins such as apolipoproteins (Morillas *et al.* 1994; Sánchez-Pozo *et al.* 1994, 1995) or immunoglobulins (Navarro *et al.* 1996; Martínez-Augustin *et al.* 1997; Navarro *et al.* 1999) and may explain why dietary nucleotides promote an optimal immune response.

Dietary nucleotides and gene expression

Two lines of evidence support the idea that dietary nucleotides may exert a direct effect on gene expression. Using isolated nuclei from the small intestine or from an intestinal epithelial cell line (IEC-18) we observed a significant effect of nucleotide availability on the hypoxanthine phosphoribosyl transferase (HPRT) gene transcription rate (Walsh *et al.* 1990). A down regulation of HPRT expression was observed when there were no nucleotides available. Thus, the enzyme responsible for nucleotide salvage is not expressed when there are no nucleotides available. Further characterization of the effect of nucleotides on HPRT expression was conducted by transfecting IEC-18 with several constructs containing deletions of the HPRT promoter and 5′ flanking sequences and placing the cells in media containing or lacking nucleotides. A region of 35 bp upstream from the HPRT gene was characterized as the specific responsible *cis*-acting element, which confers sensitivity to nucleotides (Walsh *et al.* 1990). Experiments performed afterwards resulted in the purification of a sequence-specific DNA binding protein of 66 kD, the *trans*-acting element (Walsh *et al.* 1992) with the characteristics of the type of enhancers present in other class II genes. Footprint analysis has mapped the protection from DNAase hydrolysis to a sequence of GTCTGGGT by using both affinity-purified protein and crude nuclear extracts (Walsh *et al.* 1992). Database searches have identified similar sequences of this DNA motif in other genes related to cell growth and proliferation, such as the ornithine decarboxylase gene. Thus, it is conceivable that many genes that respond to dietary nucleotides may influence cell division.

Experiments with diets containing or lacking nucleotides identical to those used for RNA studies described before, have shown that the genes for both the sodium-dependent purine nucleoside transporter (SPNT) and concentrative nucleoside transporter (CNT1) are modulated by the nucleotide content of the diet (Valdés *et al.* 2000). In the case of the purine-preferring carrier SPNT, mRNA and protein amounts, in both the liver and the small intestine, decreased when no nucleotides were in the diet. A result expected considering the lower need for purine uptake. Interestingly, it has been reported that the expression of this carrier is linked to the cell cycle and in regenerating conditions such as partial hepatectomy (Felipe *et al.* 1997). In the case of the pyrimidine-preferring carrier CNT1, we found additional levels of regulation. Thus, whereas in the liver CNT1 is regulated in a similar way to SPNT, in the intestine, a reduction in the mRNA is observed together with higher amounts of protein. These findings may be explained as a result of post-transcriptional regulation. The opposite behavior of intestine and liver regarding CNT1 expression may be a consequence of the high nucleotide biosynthetic capacity of the liver and the low capacity of the small intestine. Thus, the down-regulation in the liver occurs when no nucleotides are available, whereas there is a higher uptake in the intestine in order to compensate for the low biosynthetic capacity. The regulation of carrier expression by the diet is relevant, as nucleotide carriers participate in the uptake of drugs used in antiviral and cancer therapies.

In conclusion, it is clear that dietary nucleotides influence biosynthetic processes and modulate gene expression, at least of those genes involved in nucleotide metabolism. This high degree of regulation suggests that the uptake and metabolism of nucleotides are of great importance to a number of cell types.

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