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Uptake and utilization of nucleosides for energy repletion

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Abstract

In this paper, we report that cells undergoing metabolic stress conditions may use the ribose moiety of nucleosides as energy source to slow down cellular damage. In fact, the phosphorolytic cleavage of the N-glycosidic bond of nucleosides generates, without energy expense, the phosphorylated pentose, which through pentose phosphate pathway and glycolysis, can be converted to energetic intermediates. In this respect, nucleosides may be considered as energy source, alternative or supplementary to glucose, which may become of primary importance especially in conditions of cellular stress. In accordance with the role of these compounds in energy repletion, we also show that the uptake of nucleosides is increased when the energetic demand of the cell is enhanced. As cell model, we have used a human colon carcinoma cell line, LoVo, and the depletion of ATP, with a concomitant fall in the cell energy charge, has been induced by exclusion of glucose from the medium and pre-incubation with oligomycin, an inhibitor of oxidative phosphorylation. In these conditions of energy starvation, we show that the uptake of 2′-deoxyadenosine in LoVo cells is significantly enhanced, and that the phosphorylated ribose moiety of inosine can be used for energy repletion through anaerobic glycolysis. Our data support previous reports indicating that the phosphorylated ribose stemming from the intracellular catabolism of nucleosides may be used in eukaryots as energy source, and advance our knowledge on the regulation of the uptake of nucleosides in eukaryotic cells.

Keywords: Nucleoside uptake; Oligomycin; Energy charge; Phosphorylated pentose; Pentose phosphate pathway

1. Introduction

Phosphorylated pentose is produced intracellularly from glucose, but can be also obtained from the phosphorolytic cleavage of the N-glycosidic bond of endogenous or exogenous nucleosides. Nucleosides arising from the degradation of dietary nucleic acids must enter cells through specific transport systems and may be both catabolized or re-utilized for nucleotide synthesis. In unicellular organisms and in membranes of absorptive epithelia of multicellular organisms, the transport of (deoxy)nucleosides appears to be mediated by

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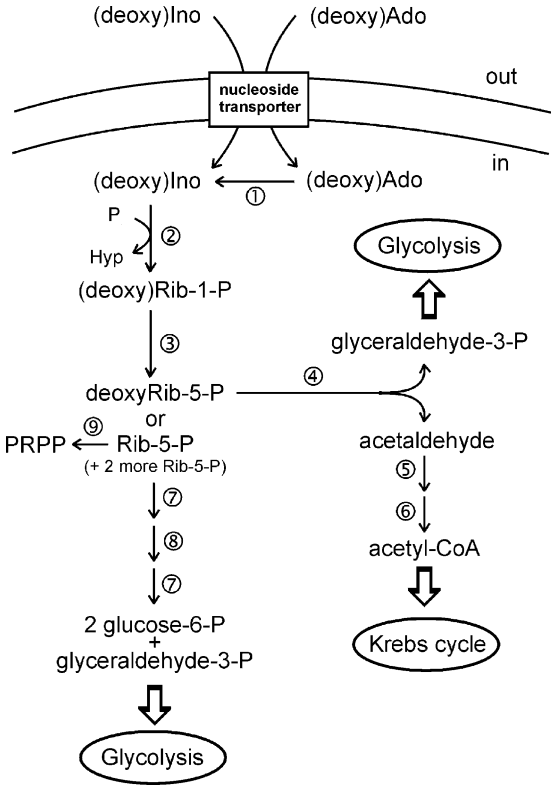
concentrative mechanisms, while in other cell types it is mediated mainly by equilibrative transport systems (Hamilton et al., 2001; Hyde, Cass, Young, & Baldwin, 2001). The phosphorolytic splitting of the N-glycosidic bond of (deoxy)nucleosides is the central catabolic reaction. By this reaction, the nucleoside structure is destroyed thus liberating (deoxy)ribose-1-phosphate [(deoxy)Rib-1-P] and the corresponding purine or pyrimidine base (Bzowska, Kulikowska, & Shugar, 2000; Pugmire & Ealick, 2002). The pentose-1-phosphate formed can be isomerized into pentose-5-phosphate. Ribose-5-phosphate (Rib-5-P) can be either utilized for 5-phosphoribosyl-1-pyrophosphate (PRPP) synthesis or, through the pentose phosphate pathway, can be converted into glycolytic intermediates. Deoxyribose-5-phosphate (deoxyRib-5-P) may undergo only a catabolic fate, being split by a specific aldolase into acetaldehyde and glyceraldehyde 3-phosphate (glyceraldehyde-3-P) (Sgarrella et al., 1997). Glyceraldehyde-3-P enters glycolysis, while acetaldehyde may be converted into acetyl-CoA by the action of two enzymes, aldehyde oxidase and acetyl-CoA synthetase (Moriwaki, Yamamoto, Yamakita, Takahashi, & Higashino, 1988). Therefore, in both cases, the pentose moiety of (deoxy)nucleosides may be utilized for energy repletion (Scheme 1).

Pioneering experiments on nucleoside metabolism demonstrated that human red cells readily catabolize inosine to hypoxanthine, while the pentose moiety is ultimately converted via the pentose phosphate pathway and glycolysis to lactate (Bartlett & Bucolo, 1968), thus leading to the net synthesis of ATP (Lionetti, 1974). Plasma inosine is the main energy source for swine and chicken erythrocytes, which lack glucose transporters (Mathew, Grdisa, & Johnstone, 1993; Young, Paterson, & Henderson, 1985). Cultured rat astrocytes subjected to combined deprivation of glucose and oxygen are protected from cell damage by adenosine and inosine (Haun, Segeleon, Trapp, Clotz, & Horrocks, 1996); purine nucleosides preserve ATP levels in glial cells during glucose deprivation and mitochondrial inhibition (Jurkowitz, Litsky, Browning, & Hohl, 1998), inosine maintains viability of mouse neuronal and glial cells during hypoxia (Litsky, Hohl, Lucas, & Jurkowitz, 1999); finally, catabolism of adenosine appears to mediate the protective effect of pre-conditioning in myocardial ischemia (Wikström, Kavianipour, Ronquist, & Waldenström, 2001). It is obvious that the metabolic



Scheme 1. Schematic illustration of the uptake and utilization of (deoxy)nucleosides. (1) Adenosine deaminase; (2) purine nucleoside phosphorylase; (3) phosphoribomutase; (4) deoxyRib-5-P aldolase; (5) aldehyde oxidase; (6) acetyl-CoA synthetase; (7): transketolase; (8) transaldolase; (9) PRPP synthetase.

fate of the pentose moiety of nucleosides may help to explain the role of these compounds in preserving different kinds of cells from energy depletion.

In a previous paper, we showed that an equilibrative transport system (ENT, equilibrative Na^+-independent nucleoside transporter) is involved in the uptake of 2′-deoxyadenosine (deoxyAdo) in a human colon carcinoma cell line (LoVo). In fact, the toxic effect exerted in LoVo cells by high concentrations of the deoxynucleoside in combination with deoxycoformycin (dCF) (an inhibitor of adenosine deaminase) was reverted by the addition in the culture medium of dipyridamole (a known inhibitor of equilibrative transporters) (Camici et al., 1995). The observation that the toxic effect of the combination was unaffected by nitrobenzylthioinosine (NBTI) (Giannecchini et al., 2003) suggests that the equilibrative transporter belongs to the ENT2