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Review

The many isoforms of human adenylate kinases

Christakis Panayiotou*, Nicola Solaroli, Anna Karlsson

Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska Institute, F68, SE-141 86 Huddinge, Sweden

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ABSTRACT

Adenine nucleotides are involved in a variety of cellular metabolic processes, including nucleic acid synthesis and repair, formation of coenzymes, energy transfer, cell and ciliary motility, hormone secretion, gene expression regulation and ion-channel control. Adenylate kinases are abundant phosphotransferases that catalyze the interconversion of adenine nucleotides and thus regulate the adenine nucleotide ratios in different intracellular compartments. Nine different adenylate kinase isoenzymes have been identified and characterized so far in human tissues, named AK1 to AK9 according to their order of discovery. Adenylate kinases differ in molecular weight, tissue distribution, subcellular localization, substrate and phosphate donor specificity and kinetic properties. The preferred substrate and phosphate donor of all adenylate kinases are AMP and ATP respectively, but some members of the family can phosphorylate other substrates and use other phosphate donors. In addition to their nucleoside monophosphate kinase activity, adenylate kinases were found to possess nucleoside diphosphate kinase activity as they are able to phosphorylate both ribonucleoside and deoxyribonucleoside diphosphates to their corresponding triphosphates. Nucleoside analogues are structural analogues of natural nucleosides, used in the treatment of cancer and viral infections. They are inactive prodrugs that are dependent on intracellular phosphorylation to their pharmacologically active triphosphate form. Novel data presented in this review confirm the role of adenylate kinases in the activation of deoxyadenosine and deoxycytidine nucleoside analogues.

Contents

Abbreviations: AK, adenylate kinase; FAD, flavin adenine dinucleotide; NAD, nicotinamide adenine dinucleotide; NMPK, nucleoside monophosphate kinase; NDPK, nucleoside diphosphate kinase; NTP, ribonucleoside triphosphate; dNTP, deoxyribonucleoside triphosphate; UMP-CMPK, uridylate-cytidylate kinase; GUK, guanylate kinase; dTMPK, thymidylate kinase; mtDNA, mitochondrial DNA; dCK, deoxycytidine kinase; dGK, deoxyguanosine kinase; dAdo, deoxyadenosine; dCyd, deoxycytidine; CdA, 2-chloro-2′-deoxyadenosine; FaraA, 2-fluoro-9-β-D-arabinofuranosyladenine; araC, 1-β-D-arabinofuranosylcytosine; dFdC, 2′,2′-difluorodeoxycytidine; TLC, thin layer chromatography.

* Corresponding author. Tel.: +46 8 58581139; fax: +46 8 58587933.
E-mail address: chrispan13@hotmail.com (C. Panayiotou).

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1. Introduction

The adenylate kinase (AK) family of isoenzymes belongs to the cellular nucleotide synthetic machinery. Nucleotides make up the structure of nucleic acids and are also important in cell metabolism either as a source of chemical energy or as activated intermediates in many biosynthetic pathways, in cell signalling and as components of coenzymes. All nucleotides need to be synthesized within the cells since there are no carrier proteins in the cell membrane and the negatively charged phosphate groups prevent diffusion across the membrane. There are two pathways for nucleotide synthesis in mammalian cells termed the *de novo* pathway and the salvage pathway, where many nucleoside and nucleotide kinases are involved. Several diseases are caused by defects in nucleotide synthesis but no or few treatments are available. Increased knowledge about the metabolic pathways for nucleotide synthesis is important for the development of both diagnostic methods and possible novel treatment strategies. In this review, we summarize the existing knowledge on the family of human adenylate kinases since during the last few years' novel enzyme isoforms have been identified and characterized.

2. Nucleotides

2.1. Functions of adenine nucleotides

All types of cells contain a wide variety of nucleotides and their derivatives, which are involved in many metabolic processes that must be carried out for normal cellular growth and function. Ribonucleotides and deoxyribonucleotides are the monomeric subunits of RNA and DNA respectively and in addition, they are components of coenzymes such as CoA, flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD) (Lodish et al., 2000). ATP is the main form of chemical energy available to the cell and it serves as the driving force of many metabolic reactions and also as a phosphate donor. Other recognized functions of adenine nucleotides involve those in which the nucleotides and their derivatives serve as mediators of key metabolic processes. cAMP is a ubiquitous intracellular second messenger, the levels of which are increased in response to a variety of hormonal and chemical stimuli. cAMP can activate the cAMP-dependent protein kinase and thereby activate or inhibit the activity of several enzymes, regulates the expression of specific genes, activates calcium channels and controls glycogenolysis and glycogenesis (Berg et al., 2002). AMP regulates a number of AMP sensitive components, including those in the glycolytic and glycogenolytic pathways and also metabolic sensors and effectors such as the K-ATP channels and AMP-activated protein kinase, which in turn can regulate cellular energy balance through managing calcium influx and phosphorylating targeted proteins (Berg et al., 2002; Dzeja and Terzic, 2009). ADP plays a key role in normal platelet aggregation and hence blood coagulation (Cattaneo and Gachet, 1999). Another important function of adenine nucleotides is their use as pharmacologically active agents in anti-viral and anti-cancer therapy.

2.2. Nucleotide synthesis

2.2.1. The de novo pathway of purine nucleotide synthesis

In proliferating cells, the *de novo* nucleotide synthesis is the main source for nuclear DNA replication and transcription. Purine nucleotides are synthesized *de novo* by a stepwise building of the ring directly on 5′-phosphoribose to form inosine monophosphate (IMP), which serves as the precursor for AMP and GMP. The ring is constructed by amino acids, N10-formyltetrahydrofolate and CO_2. AMP and GMP are then phosphorylated to their diphosphate forms ADP and GDP respectively by nucleoside monophosphate kinases (NMPKs). ADP and GDP are either further phosphorylated to their triphosphate forms (ATP and GTP) by nucleoside diphosphate kinases (NDPKs) or they are converted by ribonucleoside reductase to deoxyribonucleoside diphosphates (dADP and dGDP), which in turn are phosphorylated to dATP and dGTP by NDPKs (Reem, 1972; Reichard, 1988) (Fig. 1).

2.2.2. Salvage pathway of purine nucleotide synthesis

The salvage pathway is believed to supply quiescent or terminally differentiated cells with ribonucleoside triphosphates (NTPs) and deoxyribonucleoside triphosphates (dNTPs) necessary for DNA replication, transcription and repair. Ribonucleosides and deoxyribonucleosides, that derive either from free bases or dephosphorylation of existing NTPs and dNTPs after degradation of RNA and DNA respectively, are imported into the cells by nucleoside carrier proteins that facilitate diffusion or actively transport nucleosides across the membrane. The next step is the phosphorylation of ribo- and deoxyribonucleosides to their monophosphate form by ribonucleoside and deoxyribonucleoside kinases respectively. The nucleoside monophosphates are then phosphorylated to their triphosphate forms in two consecutive phosphorylation steps catalyzed by NMPKs and NDPKs (Arner and Eriksson, 1995).

3. Nucleoside monophosphate kinases

NMPKs are ubiquitous enzymes, present both in prokaryotes and eukaryotes that catalyze the reversible phosphoryl transfer between various nucleoside mono- and triphosphates both in the salvage and the *de novo* pathways of nucleotide metabolism. This enzyme family is further divided into subgroups consisting of adenylate kinases [EC 2.7.4.3], uridylate-cytidylate kinases (UMP-CMPK) [EC 2.7.4.14], guanylate kinases (GUK) [EC 2.7.4.8], and thymidylate kinases (dTMPK) [EC 2.7.4.9], on the basis of the differences in substrate specificity. In marked contrast to the unique phosphoryl acceptor specificity of each subgroup, the phosphate donor specificity is not so high, with ATP serving as the general phosphate donor. Three functional domains have been described in the primary structure of NMPKs: the phosphate donor binding glycine rich region (p-loop), the substrate binding site and the lid domain that closes over the substrate upon binding (Scheffzek et al., 1996).dTMPK phosphorylates dTMP and dUMP to their respective diphosphate forms (Arima et al., 1977; Su and Sclafani, 1991). The best phosphate donors for dTMPK are ATP, dATP, GTP and dGTP and none of the pyrimidine triphosphates tested could act as a phosphate donor. Thymidine was found to inhibit the activity of