

Adenylyl Cyclases

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Overview

Adenosine 3':5'-monophosphate (cAMP) modifies cell function in virtually all eukaryotic cells through the activation of cAMP-dependent protein kinase or through cAMP-gated ion channels. Cellular levels of cAMP reflect the balance between the activities of adenylyl cyclase (ATP:pyrophosphate lyase, cyclizing; EC 4.6.1.1), a family of enzymes that catalyze the formation of cAMP from 5'-ATP, and cAMP phosphodiesterases, a family of enzymes that catalyze the conversion of cAMP to 5'-AMP.

Adenylyl cyclases occur throughout the animal kingdom and play diverse roles in cell regulation. In bacteria, the enzyme may be regulated in response to nutrients or it may be secreted (for example, from *Bacillus pertussis* or *Bacillus anthracis*) and constitutes a toxic factor in mammals.

In mammals, there are at least ten distinct adenylyl cyclase isozymes, all but one of which are membrane-bound and are central to one of the most important transmembrane signal transduction pathways. The soluble form is regulated by bicarbonate, whereas membrane-bound forms are regulated by numerous neurotransmitters and hormones through cell-surface receptors linked via heterotrimeric ($\alpha\beta\gamma$) stimulatory (G $_s$) and inhibitory (G $_i$) guanine nucleotide-dependent regulatory proteins (G proteins). Most isozymes are activated by G α_s , but differ more significantly in their regulation by G α_i and in the effects of G $\beta\gamma$. These adenylyl cyclase isozymes exhibit a putative topology with 12 membrane-spanning regions and two ~40 kDa cytosolic domains (C $_1$ and C $_2$), one after each six membrane-spanning region. C $_1$ and C $_2$ share large conserved regions that interact to form a cleft form-

ing the catalytic active site. N-terminus domains are highly variable and serve regulatory roles. Activation by G α_s occurs through its interaction with the C $_2$ domain of adenylyl cyclase yielding the active enzyme: GTP• α_s •C. Inhibition by G proteins may occur by a direct effect of G α_i with the C $_1$ domain of adenylyl cyclase or by the recombination of $\beta\gamma$ with G α_s .

Adenylyl cyclase activity is also altered by numerous other agents of physiological and biochemical interest. These include agents or enzymes that act indirectly, by effects on hormone receptors, or G α_s (e.g. cholera toxin) or G α_i (e.g. pertussis toxin), and agents that act directly on the enzyme and in an isozyme-selective manner. Essentially all adenylyl cyclases are inhibited by oxidants and are protected by thiols. Most are stimulated by the diterpene forskolin, whereas regulation by calcium ions is isozyme specific.

The cleft formed by the C $_1$ •C $_2$ domains of adenylyl cyclase binds both substrate and forskolin. The active site shares topology and reaction mechanism with guanylyl cyclases, with which there is considerable homology, and with oligonucleotide polymerases. Each catalyzes a cation-dependent attack of the 3'-OH on the α -phosphate of a nucleoside triphosphate, with pyrophosphate as leaving group. Adenylyl cyclases exhibit a reversible bireactant sequential mechanism in which free divalent cation and cation-5'-ATP serve as substrates and cAMP, metal-pyrophosphate, and free divalent cation are products.

Although agents that indirectly activate or inhibit adenylyl cyclases are commonly used in the treatment of disease, e.g.

β -adrenoceptor blockers, drugs acting directly on the enzyme have been less well explored. The main classes of such agents are derivatives of either forskolin or adenine nucleotides. Probably all adenylyl cyclases are inhibited competitively by substrate analogs, the best of which is β -L-2',3'-dideoxy-adenosine-5'-triphosphate (IC $_{50}$ ~ 24 nM), and most are also inhibited by adenine nucleoside 3'-polyphosphates, the most potent of which are 2',5'-dideoxyadenosine-3'-triphosphate (IC $_{50}$ ~ 40 nM) and 2',5'-dideoxyadenosine-3'-tetraphosphate (IC $_{50}$ ~ 7 nM). These compounds belong to a class of inhibitors historically called P(purine)-site ligands, which inhibit via a noncompetitive, dead-end, post-transition state mechanism which makes them specific for adenylyl cyclases. Inhibition by these ligands is conserved with varying sensitivity in all isozymes, save those of bacteria and sperm, and they provide an exquisite means for inhibition of this signal transduction pathway.

The best cell permeable inhibitors of adenylyl cyclases include, in order of potency, 2',5'-dideoxyadenosine, 9-(tetrahydrofuryl)-adenine (SQ 22,536) and 9-(cyclopentyl)-adenine, the last being both chemically and metabolically stable. These compounds have been used to lower cellular cAMP levels and to alter function in numerous studies with both isolated cells and intact tissues.

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ISOZYME ^a (SOURCE)	I (bovine)	II (rat)	III (rat)	IV (rat)	V (rat)	VI (rat)	VII (mouse)	VIII ^d (rat)	IX (mouse)	X ^e (human) (soluble)
STRUCTURES ^b										
AMINO ACIDS	1134	1090	1144	1064	1262	1180	1099	1248(a),1218(b),1182(c)	1353	1610
ACCESSION NUMBER	M25579	M80550	M55075	M80633	M96159	M96160	U12919	L26986	U30602	AF176813
TISSUE EXPRESSION ^c	Brain	Brain	Olfactory, brain, adrenal, brown adipose	Ubiquitous	Heart, brain	Heart, kidney, brain, liver	Retina, brain	Brain	Skeletal muscle, heart, brain	Testis
Gα_s	All isozymes stimulated \longrightarrow									No effect
Gα_i		↓	↓		↓					
G$\beta\gamma$ ^f	↓	↑	No effect	↑	↓		↓			No effect
FORSKOLIN AND DERIVATIVES	Rank order of stimulatory potency: Forskolin (F 6886) > 7-Deacetyl-forskolin (D 3533) > 6-Acetyl-7-deacetyl-forskolin (A 9420) > 7-Deacetyl-7-O-hemisuccinyl-forskolin (D 4546)								No effect	No effect
CALCIUM/CALMODULIN	↑	No effect	(↓↑)	No effect	↓ (no CaM)	—	—	↑	No effect	—
PKC/PKA	↑ (PKC)	↑↑↑ (PKC)	↑ (PKC)	↓ (PKC)	↑ (PKC)	↓ (PKC) ↓ (PKA)	—	—	—	—
COMPETITIVE INHIBITORS (SUBSTRATE ANALOGS) ^g	Rank order of inhibitory potency: β -L-2',3'-dd-5'-ATP > β -D-2',3'-dd-5'-ATP > β -L-5'-ATP > 5'-APP(CH ₂)P (M 7510) \longrightarrow									
NON-COMPETITIVE INHIBITORS (P-SITE LIGANDS) ^h	Rank order of inhibitory potency: 2',5'-dd-3'-A4P > 2'-d-3'-A4P > 2',5'-dd-3'-ATP (D 0939) > 2',5'-dd-3'-ADP (D 0814) \geq 2'-d-3'-ATP > 2',5'-dd-3'-AMP (D 0689) \geq 2'-d-3'-ADP \geq 2',5'-dd-Ado (D 7408) \geq 9-Xyl-Ado > 2'-d-3'-AMP (D 3139) > 3'-ADP > 3'-AMP (A 9272 , A 0386) > 2'-d-Ado (D 7400) = 3'-Ado (C 3394) > 5'-d-Ado (D 1771) > 9-Ara-Ado (A 5762) = 9-THF-Ado (S-153) \geq 9-CP-Ado (C 4479) > Ado (A 9251)									
OTHER INHIBITORS	Rank-order of inhibitory potency: PME-App = PMEAp(NH)p > polyadenylate > 2',3'-dd-Ado (D 1285) = 2-Cl-2',3'-dd-Ado > PMEA > MDL-12,330A (M-182) \longrightarrow									

ABBREVIATIONS

Ado: Adenosine
2'-d-Ado: 2'-Deoxyadenosine
3'-d-Ado: 3'-Deoxyadenosine (Cordycepin)
5'-d-Ado: 5'-Deoxyadenosine
2',5'-dd-Ado: 2',5'-Dideoxyadenosine
2',3'-dd-Ado: 2',3'-Dideoxyadenosine
9-CP-Ado: 9-(Cyclopentyl)-adenine

9-THF-Ado: 9-(Tetrahydrofuryl)-adenine (SQ 22,536)
9-Ara-Ado: 9-(Arabinofuranosyl)-adenine
9-Xyl-Ado: 9-(Xylofuranosyl)-adenine
2'-d-3'-AMP: 2'-Deoxyadenosine-3'-monophosphate
2'-d-3'-ADP: 2'-Deoxyadenosine 3'-diphosphate
2'-d-3'-ATP: 2'-Deoxyadenosine 3'-triphosphate
2',5'-dd-3'-AMP: 2',5'-Dideoxyadenosine 3'-monophosphate
2',5'-dd-3'-ADP: 2',5'-Dideoxyadenosine-3'-diphosphate
2',5'-dd-3'-ATP: 2',5'-Dideoxyadenosine 3'-triphosphate

2',5'-dd-3'-A4P: 2',5'-Dideoxyadenosine 3'-tetraphosphate
5'-APP(CH₂)P: Adenosine 5'-(β -methylene)-triphosphate
 β -L-5'-ATP: β -L-Adenosine 5'-triphosphate
 β -L-2',3'-dd-5'-ATP: β -L-2',3'-Dideoxyadenosine 5'-triphosphate
CaM: Calmodulin
MDL-12,330A: *cis*-N-(2-Phenylcyclopentyl)azacyclotridec-1-en-2-amine
PMEA: 9-(2-Phosphonylmethoxyethyl)-adenine
PMEApp: 9-(2-Diphosphorylphosphonylmethoxyethyl)adenine

FOOTNOTES

Empty cells indicate no information currently available.

a Isozyme source is for structure information.

b Structures available at <http://www.ncbi.nlm.nih.gov>.

c Tissue expression is for evidence of protein expression; this is not a complete listing.

d Type VIII exhibits three splice variants (A,B,C); accession number is for splice variant A.

e Type X adenylyl cyclase is stimulated by bicarbonate.

f Effects of G $\beta\gamma$ are on G α_s stimulated enzyme.

g Competitive inhibitors interact with the pre-transition configuration of the enzyme. Isozyme selectivity has not been determined; comparisons are from experiments with a detergent-extract from rat brain.

h Inhibitory P-site ligands are characteristically adenine nucleosides or nucleotide phosphates that inhibit adenylyl cyclases by a noncompetitive dead-end (post-transition-state) mechanism. This comparison is from experiments with a detergent-extract from rat brain. These ligands likely inhibit all isozymes, although potencies may vary; comparisons have been made only with types I, II, VI, VII and VIII.