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Sequence homologies between guanylyl cyclases and structural analogies to other signal-transducing proteins

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The cyclic GMP-forming enzyme guanylyl cyclase exists in cytosolic and in membrane-bound forms differing in structure and regulations. Determination of the primary structures of the guanylyl cyclases revealed that the cytosolic enzyme form consists of two similar subunits and that membrane-bound guanylyl cyclases represent enzyme forms in which the catalytic part is located in an intracellular, C-terminal domain and is regulated by an extracellular, N-terminal receptor domain. A domain of 250 amino acids conserved in all guanylyl cyclases appears to be required for the formation of cyclic nucleotide, as this homologous domain is also found in the cytosolic regions of the adenylyl cyclase. The general structures of guanylyl cyclases shows similarities with other signal transducing enzymes such as protein-tyrosine phosphatases and protein-tyrosine kinases, which also exist in cytosolic and receptor-linked forms.

Guanylyl cyclase; Protein-tyrosine kinase; Protein-tyrosine phosphatase; Adenyl cyclase; Transmembrane signalling

1. INTRODUCTION

The cyclic nucleotides, cyclic AMP and cyclic GMP, are intracellular signal molecules involved in communications within cells. Although cyclic GMP was discovered more than 20 years ago, the understanding of its physiological functions was very limited for a long time. Now there is evidence for regulatory roles of cyclic GMP in smooth muscle tone and platelet function [1]. The effects of cyclic GMP in these and other systems are mainly mediated by cyclic GMP-stimulated protein kinases and possibly by cyclic GMP-modulated cAMP phosphodiesterases. Cyclic GMP-sensitive cAMP phosphodiesterases are either stimulated or inhibited by cyclic GMP; hormones and drugs which increase cyclic GMP levels can, thereby, have either synergistic or antagonistic effects with cAMP-elevating hormones, depending on whether cyclic GMP-inhibited or cyclic GMP-stimulated cAMP phosphodiesterases are present in a given cell type. In the vision system of vertebrates, cyclic GMP plays a key role by direct regulation of a cation channel, which is kept open in the darkness by high cyclic GMP, acting as a channel ligand, and which closes during visual excitement as a result of an increased cyclic GMP hydrolysis triggered by illumination [2]. The existence of cyclic GMP-regulated ion channels appears not to be limited to the retina, as cyclic GMP-regulated ion channels have also been demonstrated in renal collecting ducts, where increasing cyclic GMP levels reduce Na^+ reabsorption by inhibiting an amiloride-sensitive cation channel, an effect which is partially direct by a phosphorylation-independent mechanism and partially indirect through activation of cyclic GMP-dependent protein kinase [3]. The diarrhea induced by heat-stable enterotoxin from *E. coli*, which is known to elevate cyclic GMP levels in the intestinal epithelia (for refs. see [1]), is probably also caused by cyclic GMP changing the open probability of ion channels.

In contrast to the formation of cyclic AMP, which in higher organisms is restricted to the plasma membrane, biosynthesis of cyclic GMP occurs in cytosolic and particulate cell fractions. Most tissues contain both enzyme forms, but the relative distribution varies from tissue to tissue, with extraordinary situations in intestinal mucosal cells, where most of the guanylyl cyclase is bound to plasma membranes, and in platelets, where almost all of the enzyme is cytosolic (for refs see [1,4]). Some structural properties and activators of cytosolic and membrane-bound guanylyl cyclases are summarised in Table I.

2. REGULATION OF CYTOSOLIC GUANYLYL CYCLASE

Since the early seventies, regulations of intracellular cyclic GMP concentrations have been demonstrated by various hormones and neurotransmitters in intact cells (for refs see [1]). The continued failure of hormonal stimulation of the enzyme in broken cell preparations led to the hypothesis that cytosolic guanylyl cyclase is regulated by hormonal factors through indirect mechanisms, e.g. by intermediates of the Ca^{2+}- and O_2-dependent biosynthesis of eicosanoids [5]. In the

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Table I

Guanylyl cyclases: principal structures and modulations

Subcellular localization	Activators	Source	Properties	Structure	Molecular mass		Cyclase domains	Transmembr. domains
					SDS-gels	cDNA-deduced		
Cytosol	NO, R-NO	mammalian tissues						
		bovine lung	hemoprotein	dimer	70 + 73	70.5 + 77.5	2	-
		rat lung	?	dimer	70 + 82	70.5 + 77.5	2	-
		rat kidney	?	?		? + 76.3		
Plasma membrane	type A: ANP > BNP	mammalian tissues	glycoprotein	monomer	120-180	115	1	1
	type B: BNP > ANP	mammalian tissues	glycoprotein ?	monomer		114	1	1
	resact	sea urchin sperm	glycoprotein	monomer	135	123	1	1
	type C: ST	mammalian intestine	glycoprotein ?	monomer		121	1	1
	Ca^{2+} (↓)	mammalian retina						
Plasma membrane	Ca^{2+} (↑)	Paramecium Tetrahymena Dictyostelium						

ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; ST = heat-stable enterotoxin of *E. coli*; ↑,↓ = increased or decreased activity by Ca^{2+}, respectively.

late seventies, NO-containing compounds were demonstrated to be potent activators of cytosolic guanylyl cyclase [6,7]. As the cytosolic enzyme form was characterized as a heme-containing protein, it was proposed that NO-containing compounds or liberated NO interact with the heme component, thereby stimulating the enzyme [8]. Nevertheless, no physiological activators of the cytosolic guanylyl cyclase were identified, until vasodilatory factors such as acetylcholine, histamine and bradykinin were shown to induce the formation of a labile substance distinct from prostacyclin in endothelial cells ([9] and refs therein). This compound, called endothelium-derived relaxing factor (EDRF), stimulates cytosolic guanylyl cyclase and causes vasodilation via elevated cyclic GMP concentrations. EDRF was identified as NO or as an NO-containing compound (R-NO) [10], which is formed in a Ca^{2+}/calmodulin-, NADPH- and tetrahydrobiopterin-dependent manner from L-arginine [11-13]. Therefore, an increased cytoplasmaic Ca^{2+} concentration appears to be the hormonally induced signal causing a subsequent increase in R-NO synthesis and stimulation of cytosolic guanylyl cyclase. As the biosynthesis of R-NO is not restricted to endothelial cells but has also been demonstrated in various other tissues, it is now assumed that NO or R-NO is a widespread signal molecule with cytosolic guanylyl cyclase as an effector system.

3. REGULATION OF MEMBRANE-BOUND GUANYLYL CYCLASES

Research on membrane-bound guanylyl cyclase was greatly stimulated by the discovery that peptide hormones activate the membrane-bound form of guanylyl cyclase but not the cytosolic one. The membrane-bound guanylyl cyclase of sea urchin sperm was shown to be stimulated by the chemotactic peptide resact, whereas mammalian membrane-bound guanylyl cyclases are stimulated by atrial natriuretic (ANP) and related peptides (for refs see [4]). The effect of ANP can be markedly potentiated by the addition of ATP or its analogue adenosine-5'-*O*-(3'-thiotriphosphate) [14]. Isolation of the membrane-bound guanylyl cyclase resulted in copurification with an ANP receptor subtype and suggested a rather tight coupling between the membrane-bound guanylyl cyclase and the ANP receptor (for refs see [1]).

A membrane-bound guanylyl cyclase, which is activated by the heat-stable enterotoxin of *E. coli*, occurs in the intestinal mucosa (for refs see [1]). No physiological activator of the enzyme is known. The occurrence of the heat-stable enterotoxin-activated guanylyl cyclase may not be limited to intestinal mucosa, as in kidneys of opossum and kangeroo and in certain colon carcinoma cells the heat-stable enterotoxin leads to elevation of cyclic GMP levels [15]. The

characterisation of enterotoxin-activated guanylyl cyclase is very poor, as attempts to purify the enzyme have failed so far.

Retinal rod outer segments contain a membrane-bound guanylyl cyclase, which in contrast to other guanylyl cyclases is activated by decreasing concentrations of Ca^{2+}. Visual excitation in retinal rod cells is mediated by a cascade of events which lead to increased hydrolysis of cyclic GMP and the consequent closure of cyclic GMP-activated cation-specific channels, whereby the influex of Ca^{2+} and other cations is blocked. The lowering of cytosolic Ca^{2+} stimulates the retinal guanylyl cyclase, and through the resynthesis of cyclic GMP the dark state is recovered. The inhibitory effect of Ca^{2+} on guanylyl cyclase activity is mediated by a Ca^{2+}-sensitive regulatory protein (for refs see [2]).

Another Ca^{2+}-regulated form of a membrane-bound guanylyl cyclase has been found in Paramecium and Tetrahymena plasma membranes. In contrast to the effect on the enzyme in retina, Ca^{2+} leads to stimulation of this enzyme; the Ca^{2+}-sensitive protein mediating the response to the cation appears to be calmodulin (for refs see [1]); however, Ca^{2+}/calmodulin-activated guanylyl cyclases have so far not been detected in vertebrates.

4. STRUCTURES OF CYTOSOLIC AND MEMBRANE-BOUND GUANYLYL CYCLASES

Cytosolic guanylyl cyclase was purified to apparent homogenity in several laboratories and was shown to consist of two different subunits. Two groups developed immunoaffinity purification procedures, which yielded enzymes with subunits of 70 and 82 kDa from rat lung [16] and 70 and 73 kDa from bovine lung [17] (apparent molecular masses on SDS-PAGE). Sequence analyses of the 73 kDa [18] and 82 kDa [19] subunit revealed that both subunits consist of 691 amino acids, have a calculated molecular mass of 77.5 kDa and share 84.6% of the amino acids. The variations observed in the sequences are probably due to the differences in species, although the bovine [20] and rat [21] 70 kDa subunits are more conserved, showing 97.4% identical amino acids. A comparison of the amino acid sequences of the bovine 70 kDa and 73 kDa subunit revealed strong similarities between the subunits with about 32% identical amino acids over the whole sequences [18]. The homology is most prominent in the central part of the molecules and continues towards the C-terminus. In the N-terminal part of the sequences, which show rather low homology, four conserved cysteine residues are found, which may be important for redox regulation of the enzyme. Expression experiments with soluble bovine and rat guanylyl cyclase subunits revealed that coexpression of both subunits is necessary for the catalytic function of the enzyme [19,22]. This finding and the high structural homology between the two subunits suggest that both subunits are involved in the formation of cyclic GMP. Very recently, the sequence of another cytosolic guanylyl cyclase subunit, which is preferentially expressed in rat kidney, was reported [23]. The existence of different forms of subunits demands a unifying and consistent subunit nomenclature. As proposed by Garbers and associates [23], the subunits of soluble guanylyl cyclase (GC-S) should be referred to as α and β subunits, whereby α_1 refers to the 73/82 kDa subunit (apparent molecular masses on SDS-PAGE) and β_1 to the 70 kDa of the enzyme characterised from lung. The new subunit [23], which was identified using the polymerase chain reaction with oligonucleotides corresponding to amino acid sequences conserved between all guanylyl cyclases, has a calculated molecular mass of 76.3 kDa and shows the highest degree of similarity to the β_1 subunit with 34% of identical amino acids over the whole length of the sequences. This new subunit, designated β_2, lacks the first 62 amino acids of the β_1 subunit but extends with C-terminal 86 amino acids beyond the C-terminus of β_1 and contains a consensus sequence for isoprenylation/carboxymethylation [23]. The diversity in subunits of soluble guanylyl cyclase suggests possible differences in regulations. As the expression of the β_2 subunit by itself did not yield an enzymatically active guanylyl cyclase, the question remains whether the β_2 subunit can form a catalytically active heterodimer with the α_1 subunit or whether another α subunit is necessary for catalytic activity. In addition the mechanism of activation of the resulting heterodimer has to be clarified.

In contrast to the cytosolic guanylyl cyclase, membrane-bound guanylyl cyclases consist of one subunit with a M_r of about 120–180 kDa. All membrane-bound enzymes purified so far are glycosylated. The mammalian enzyme was shown to bind ANP; however, this binding was not associated with increased enzymatic activity of the purified enzyme (for refs see [4]).

The primary structures of two mammalian, type A and type B, membrane-bound guanylyl cyclases and a membrane-bound guanylyl cyclase from sea urchin sperm have been determined (for refs see [4]). The amino acid sequences predict proteins that are structurally related to the protein-tyrosine kinase-linked growth factor receptors, with an N-terminal extracellular part, one trans-membrane domain and a C-terminal cytosolic region (Fig. 1). The membrane-bound guanylyl cyclases show weak homology to the cytosolic region of the growth factor receptors, which is supposed to be responsible for the tyrosine kinase activity; for membrane-bound guanylyl cyclases tyrosine kinase activity has, however, not been demonstrated. The N-terminal parts of the ANP-stimulated guanylyl cyclases show homologies to another, smaller ANP receptor which is not coupled to a guanylyl cyclase, and

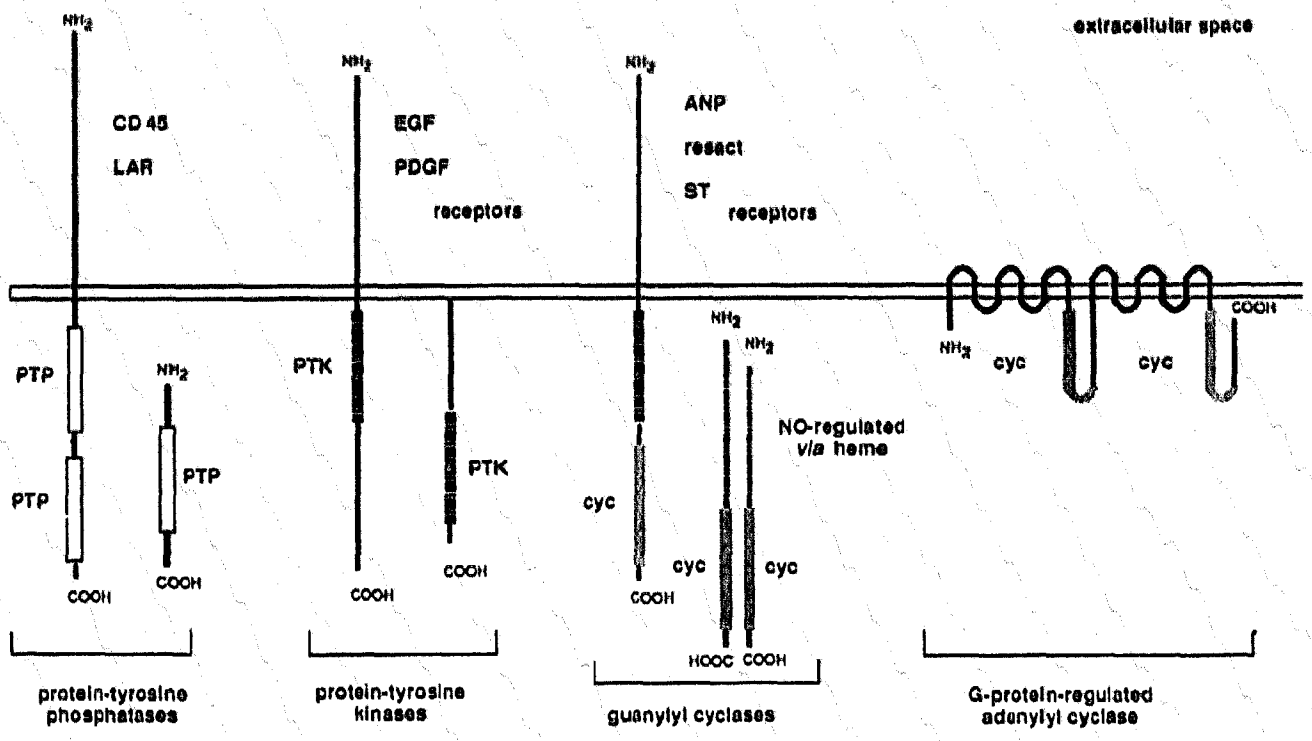

Fig. 1. Schematic representation of structurally related enzyme families. Shown are the membrane-bound and cytosolic forms of protein-tyrosine phosphatases, protein-tyrosine kinases, guanylyl cyclases and the membrane-bound adenylyl cyclase. Labelled boxes indicate sequence homologies. PTP, putative catalytic domain of protein-tyrosine phosphatases; PTK, putative catalytic domain of protein-tyrosine kinases; cyc, putative catalytic domain of guanylyl and adenylyl cyclases; CD 45, leukocyte common antigen; LAR, leukocyte common antigen-related molecule; EGF, epidermal growth factor; PDGF, platelet derived growth factor; ANP, atrial natriuretic peptide; ST, heat-stable enterotoxin of *E. coli*. For further explanations see text.

the C-terminal region of all membrane-bound guanylyl cyclases contains a stretch of about 250 amino acids homologous with the subunits of the cytosolic enzyme (see below). Expression of the mammalian membrane-bound guanylyl cyclases proved that the proteins serve as a receptor for ANP and contain guanylyl cyclase activity and that catalytic activity is increased by hormone binding (for refs see [4]). Deletion of the region homologous to the tyrosine kinases resulted in a permanent ANP- and ATP-independent activation of membrane-bound guanylyl cyclase (for refs see [4]).

The ANP-stimulated guanylyl cyclases type A and B share an overall homology of 62% identical amino acids; the intracellular regions appear more highly conserved than the extracellular domains (78% versus 43% identity). This may explain the differences in potencies of various natriuretic peptides on guanylyl cyclase A and B activities; the relatively high ANP concentrations required to elicit a response of the guanylyl cyclase B suggests the possible physiological stimulation by a more potent, unidentified natural ligand (for refs see [4].

A comparison of the sequences of the subunits of the cytosolic guanylyl cyclase and the sequences of all membrane-bound guanylyl cyclases sequenced so far revealed a homologous domain of about 250 amino acids in the C-terminal regions of all molecules [18]. Two of these homologous domains are also found in the cytosolic parts of adenylyl cyclase from bovine brain [24] and rat olfactory neurons [25] (see Fig. 1). The primary structures of the sequenced mammalian adenylyl cyclases predict proteins with 12 membrane spanning regions and two large hydrophilic domains, which are similar to each other and share homologies with the C-terminal part of all guanylyl cyclases [18]. As the regulation of all these cyclases is very different (coupling to G-proteins, direct hormone binding and NO-heme interaction for adenylyl cyclase, plasma membrane-bound and cytosolic guanylyl cyclases, respectively), but the principal reaction catalyzed by the enzymes is the same, we presume that the homologous region common to all sequences represents a domain involved in the catalytic function of the cyclases.

Out of the adenylyl cyclases of lower organism, i.e. bacteria and yeast, sequenced so far ([26] and refs therein), only the adenylyl cyclase of yeast shows some rather low homologies to the hydrophilic domains of the mammalian adenylyl cyclase; the homologies to guanylyl cyclases are even less. Among the group of adenylyl cyclases of lower organism, only the

calmodulin-sensitive adenylyl cyclases from *Bacillus anthracis* and from *Bordetella pertussis* show significant homologies between each other.

5. STRUCTURAL SIMILARITIES OF THE GUANYLYL CYCLASES TO THE FAMILIES OF PROTEIN-TYROSINE KINASES AND PROTEIN-TYROSINE PHOSPHATASES

Occurrence of the membrane-bound guanylyl cyclases type A and B and the resact-receptor coupled guanylyl cyclase with highly conserved intracellular domains but relatively distinct receptor domains suggests the existence of more isoforms of guanylyl cyclases, which may all share the catalytic domain, but are linked to different receptor domains and, thereby, are activated by different ligands. In the course of the preparation of this review, Garbers and associates published the primary structure of another membrane-bound form of guanylyl cyclases (GC type C), which had been obtained by the polymerase chain reaction using template cDNA from small intestine mucosa [27]. The sequence revealed strong similarities to the other membrane-bound guanylyl cyclases in the intracellular part of the molecule while the extracellular ligand binding domain showed almost no homologies. The cyclic GMP-forming activity of the expressed membrane-bound guanylyl cyclase type C was enhanced by the preincubation with the enterotoxin of *E. coli*. These data demonstrate that indeed the guanylyl cyclase stimulated by the enterotoxin of *E. coli* is an isoform of the membrane-bound guanylyl cyclases type A and B with a different hormone binding domain.

The principle of coupling various extracellular receptor domains to a common intracellular catalytic domain and the existence of a cytosolic enzyme form containing a homologous catalytic domain appears to be analogous to the families of protein-tyrosine kinases [28] and protein-tyrosine phosphatases [29]. In the membrane-bound form of tyrosine kinases, the catalytic intracellular domain shared by all members of the family is coupled to different growth factor receptors represented by the extracellular part of the molecules. The cytosolic enzyme forms, e.g. the c-src protoncogene product $pp60^{c-src}$, are not coupled to a receptor, and the mechanism of regulation appears to be very complex and is not fully understood. In the leukocyte common antigen, CD45, which comprises a broad family of structurally related membrane-spanning molecules in hematopoietic cells, a tandem repeat of the putative protein-tyrosine phosphatase domain is linked to large, different external N-terminal domains. In the leukocyte common antigen-related molecule (LAR), again two catalytic phosphatase domains are connected to an external domain displaying structural characteristics of the N-CAMs. Nothing is known about the ligand of CD45, but N-CAM is known to be involved in cell-cell interaction, and such interaction could modulate the activity of the C-terminally located tyrosine phosphatase. The cytosolic form of the protein-tyrosine phosphatases contains only one catalytic domain, whose regulation is unknown.

6. CONCLUSIONS

Analysis of the sequences of different guanylyl cyclases reveals one region of about 250 amino acids with strong homologies, which probably represents the catalytic domain as this homologous region is also found in the adenylyl cyclase, an enzyme whose regulation is quite different from that of the guanylyl cyclases but which in principle catalyzes the same reaction, i.e. formation of a cyclic nucleotide from nucleoside triphosphate. The fact that cytosolic guanylyl cyclase and adenylyl cyclase contain two putative catalytic domains suggests that two of these domains are necessary for enzymatic activity of the cyclases, but dimerisation of the membrane-bound guanylyl cyclase has not been demonstrated. This hypothesis is supported by the finding that receptor-linked tyrosine kinases, which are structurally related to the membrane-bound guanylyl cyclases, undergo dimerisation in the process of hormonal stimulation [30]. Besides the sequence homology to the adenylyl cyclases, the family of guanylyl cyclases shows structural analogies to the families of the protein-tyrosine kinases and protein-tyrosine phosphatases. All of these enzyme families consist of membrane-bound enzyme forms, in which different extracellular receptor domains are linked to a common catalytic domain, and of cytosolic enzyme forms containing a homologous catalytic domain. As the number of protein-tyrosine kinases and protein-tyrosine phosphatases has increased dramatically during the last years, the family of guanylyl cyclases is also expected to grow significantly in the near future.

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