The sequence of the hemoregulatory peptide is present in $G_{i\alpha}$ proteins

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The hemoregulatory peptide PyroGlu-Glu-Asp-Cys-Lys (HP5b), which inhibits myelopoietic colony formation in vitro, is shown to be a sequence motif which is also part of the effector domain of $G_{i\alpha}$ proteins. Out of 8 synthetic peptides with sequence variations of HP5b, those with the closest similarity to the $G_{i\alpha}$ sequence are biologically active. The inhibitory effect appears to be dependent on the blocked N-terminus. It is postulated that these peptides may interfere with signal transduction mediated by $G_{i\alpha}$ proteins.

G-protein; Hemopoiesis; Hemoregulatory peptide

1. INTRODUCTION

The hemoregulatory peptide (HP5b) is an acidic pentapeptide which inhibits myelopoietic cell proliferation with high selectivity [1–4]. It seems to be secreted by mature granulocytes both in human and in animals [5]. The peptide may be the part of a general regulatory system mediated by negative feedback mechanisms, since structurally related peptides have a similar selective inhibitory effect on epidermal cells [6] and hepatocytes [7].

Although the peptide is taken up by hemopoietic cells [8] and there is some evidence for a cell receptor [5], nothing is known about receptor physiology, internalization or signal transduction mediated by the molecule. During the search for other active compounds, a series of peptides structurally related to the original HP5b were synthesized chemically. We report here that biologically active peptides have a close similarity to a five amino acid sequence in the effector domain of the α-subunit of three sequenced inhibitory G-proteins.

The initial reason to search for protein sequences with homology to the hemoregulatory peptide was born out of the question whether such a small peptide could be encoded by a gene, or whether it would be generated by processing a larger polypeptide precursor. If such a precursor would have a different function in the cell, it might have been discovered already in a different context and could be retrieved from the current data bases. A second aspect of the search was of course the finding that sequence motif in perhaps well-characterized proteins might provide us with hints how the hemoregulatory peptide could possibly function.

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2. MATERIALS AND METHODS

2.1. *Materials*

Altogether seven pentapeptides were synthesized as earlier described [5,8] and, following repurification, were obtained as 95–99% pure crystalline material (courtesy by Nycomed, Oslo). The peptides were dissolved in 0.9% NaCl containing 5×10^{-5} M Ca Na_2 EDTA, or 10^{-4} M mercaptoethanol to avoid dimerization.

2.2. *Hemopoietic cell culture*

A one-layer method with 10^5 C3H mouse bone marrow cells plated in 0.35% agar in Eagle Dulbecco's medium with 25% fetal calf serum and with mouse endotoxin stimulated serum as colony stimulating activity, was used as described earlier [3]. The numbers of colonies (over 50 cells) were counted after 7 days at 37°C and in 5% CO_2 in air.

2.3. *Computer methods*

The pentapeptide sequences were searched with FastP against the MIPSX protein sequence data base of the Max-Planck-Institute for Biochemistry, Martinsried, FRG. The library contained at the time of the search 9807 entries with a total of 2457508 residues. The maximal possible score for the pentapeptide EEDCK was 29 using the Dayhoff MD matrix. The average score was 8.3 with a standard deviation of 6.62. The top score (bovine guanine nucleotide binding protein) was 28. The search was based on dipeptide matches (2-k-tup). With 1-k-tup the search yielded the same two G-proteins as top scorers. The mean score then was 11.8 with a standard deviation of 4.89.

3. RESULTS

3.1. *Hemoregulatory peptide sequence occurs in G-proteins*

The sequence Glu-Glu-Asp-Cys-Lys was searched against the MIPSX data base using the FastP program [9]. This sequence is that of the synthetic peptide HP5b. It was chosen as search sequence because the sequence of the natural pentapeptide has not been deter-

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Table I

Homology between the $G_{i\alpha}$ subunit of G-protein and hemoregulatory peptide analogs

	$G_{i\alpha}$-protein															
Amino acid number	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
										Alternative sequences:						
	Lys	Ile	Ile	His	Glu	Asp	Gly	Tyr	Ser						Gln	Tyr ...
							bovine	$G_{i\alpha 1}$		Glu	Glu	Glu	Cys	Lys		
							rat	$G_{i\alpha 2}$		Glu	Glu	Glu	Cys	Arg		
							human	$G_{i\alpha 3}$		Glu	Asp	Glu	Cys	Lys		
							Hemoregulatory peptide analogs (HP)									
Codes:							HP5a			pGlu	Asp	Asp	Cys	Lys		
							HP5b			pGlu	Glu	Asp	Cys	Lys		
							HP5c			Glu	Glu	Asp	Cys	Lys		
							HP5d			pGlu	Glu	Glu	Cys	Lys		
							HP5e			pGlu	Asp	Glu	Cys	Lys		
							HP5f			pGlu	Glu	Asp	Cys	Arg		
							HP5g			pGlu	Glu	Glu	Ser	Lys		
							HP5h			pGlu	Glu	Glu	Cys	Arg		

mined unambiguously for positions 2 and 3 where both Asp and Glu are equally probable, and since HP5b is the most active peptide, we reasoned that it has a good chance to be equivalent to the natural sequence. The best score was found with the bovine G_i α-protein containing the sequence Glu-Glu-Glu-Cys-Lys. The second best score and with respect to human sequences the best match was also a G_i α-protein ($G_{i\alpha 3}$) with two conservative exchanges in positions 2 and 3 (Table I). In general, the sequence appears to be rare in nature, which strengthens the significance of the similarity to G-proteins. This is clearly due to the presence of the Cys residue. When we searched for Glu-Glu-Asp-Ser-Lys, the sequence of a synthetic peptide which we tested also in vivo and which is not active (see below), we found two perfect matches in the same data base.

3.2. *Biological effects*

The biological activity of several synthetic peptide variants was tested (Fig. 1, Table I). HP5a with the sequence 2-Asp-3-Asp, that was first described by Paukovits et al. 1980 [10] turned out to be inactive. The same was the case with only Glu as N-terminal residue, which also occurs when the PyroGlu-ring is split enzymatically [11]. Replacement of Cys with Ser also led to a complete loss of inhibition of CFU-GM cells (HP5g). This shows that Cys is vital for the activity of the peptide. All other sequences had an inhibitory effect on myelopoietic colony formation, partly exhibiting a characteristic bell-shaped curve. This shows that conservative exchanges such as Glu-Asp or Lys-Arg are tolerated to some extent. However it appears that there are quantitative differences in activity. For instance HP5h which was synthesised to match the sequence in rat $G_{i\alpha 2}$ appeared to be an active compound and in general the more the sequence resembles that of the G proteins, the higher the activity appears to be. No direct toxicity was found when bone marrow cells were exposed to the tested peptide concentrations in suspension culture in Eagle Dulbecco's medium for 1–24 h as tested by the Trypan blue exclusion assay and total cell counts.

4. DISCUSSION

It is known that the various classes of the G-proteins are involved in many cellular processes [12–14]. They are also active in hemopoietic cells. For example, the G_i protein has recently been localized in human neutrophils [15]. Based on indirect studies, there is also evidence that hemopoietic progenitor cell proliferation as well as interleukin-3 and CSF-1 action are mediated through the same mechanism [16,17]. On this background it is remarkable that the synthetic analog oligopeptides with structural homology to G-proteins are able to inhibit myelopoietic colony formation.

To assess the biological significance of this finding one has to consider the putative role of this sequence for the function of the $G_{i\alpha}$-proteins. This sequence at positions 63–68 is well conserved among the three $G_{i\alpha}$-proteins sequenced thus far but not found to that extent in all other G- or G-like proteins. This sequence is immediately adjacent to the well-conserved Halliday region A which has been implicated by three-dimensional analysis in p21 ras and EF-Tu as the phosphate binding domain. Since this sequence is not conserved in the well-characterized small GTP-binding proteins, little can be said about its context in the three-dimensional structure. From its position and its polar composition one can only guess that it might be an ex-

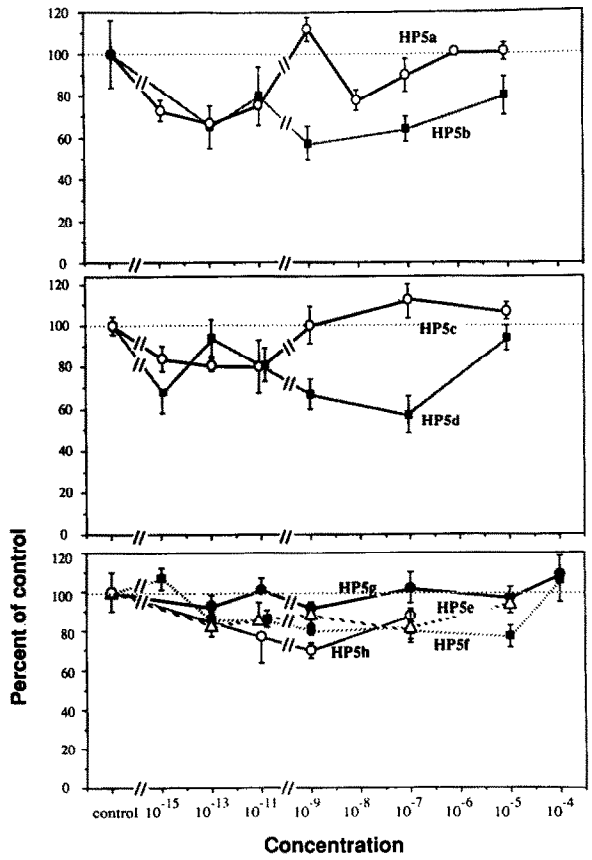

Fig. 1. Effects of the different peptides (HP5a–g) on myelopoietic colony formation (CFU-GM) expressed as percent of controls ± SE.

posed helical loop near the phosphate binding cleft. Furthermore, because of the variability of this N-terminal region in G-like proteins, the region between Halliday A and C domains is thought to be involved in effector interactions to account for the specific role of each G-protein.

It is remarkable that the hemoregulatory factor consists of a sequence that can be found in G-proteins. Since the homology is confined to $G_{i\alpha}$-proteins, it might suggest a possible way for functioning of the peptide. Peptides of that size can efficiently mimic a binding site for another protein as it was shown for the synthetic pentapeptide from laminin which binds efficiently to the laminin receptor and competes with laminin [18]. On the other hand it has been shown that binding of an oligopeptide can interfere with signal transduction. The wasp toxin mastoparan activates a G-protein presumably because its sequence resembles that of the binding site of the receptor [19]. Since hemoregulatory peptide resembles the G-protein part that binds to an effector (or receptor), it could for instance interfere with G-protein-effector binding and thus override a signal coming from a receptor through the G-protein. Alternatively, the peptide could directly activate the effector by mimicking the active G-protein-effector complex. In this case the peptide regulator would bypass the normal signal mediated through receptor/G-protein interaction.

It seems conflicting with the model we propose here that the best homology is not found with a human $G_{i\alpha}$ protein, but with that of rat, although $G_{i\alpha 3}$ can be connected to the hemopoietic system. Its amino acid sequence has been deduced from the nucleotide sequence of a human cDNA clone isolated from a differentiated HL-60 cDNA library [20]. A G-protein has also been demonstrated in erythrocyte G_{k1} where it is involved in receptor regulated K^+-channels [21]. It is not unlikely in view of the large number of existing G-proteins that another G-protein in hemopoietic precursor cells is identical to the rat protein. On the other hand for the mechanism we envisage here it is not necessary that the oligopeptide is identical to the G-protein sequence. A change in one amino acid could increase the binding of the peptide to its target relative to that of the G-protein. The consequence would be that very low concentrations of the peptide would be able to interfere with signal transduction.

It should also be noted that the analog peptides are not only acting on hemopoietic cells, although they respond with a high degree of selectivity in the lower dose range. Thus, a variety of other cell types are inhibited, although to a lesser degree and at higher concentrations [22]. This could further indicate that the peptides are either part of or are able to interfere with a general principle for proliferative regulation.

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