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STRUCTURAL FEATURES OF AMPHIPATHIC PEPTIDES REQUIRED FOR THE ACTIVATION OF G-PROTEINS

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ABSTRACT

Eight different amphipathic peptides were tested as modulators of GTPase activity of G-proteins from rat brain cortex membranes: mastoparan and melittin (components of wasp and bee venom, respectively), MAS17 (inactive mastoparan analog), M252 and M256 (peptides derived from nerve growth factor receptor), PD_1 (synthetic peptide detergent), M366 (peptide derived from β-amyloid protein) and cys-pAntp (homeodomain part of *Drosophila* antennapedia protein). Four of the peptides (mastoparan, melittin, PD_1 and M366) increased GTPase activity, other peptides showed no effect. Correlation of these data with peptide sequences, their predicted secondary structure and residue solvent accessibility pointed to two types of activators. First type (melittin and PD1) is characterised by longer (24-26 amino acids) fully amphipathic helical structure with separated charges at both ends of the sequence. Second type of activators (mastoparan and M366) is a shorter helix (11-14 amino acids) and contains a motif consisted of lysine in position 4, followed by 5 to 6 amino acids with the residues of low solvent accessibility.

INTRODUCTION

It is well known that several amphipathic peptides activate G-proteins. It was suggested that mastoparan, a component of wasp venom, and some of its analogs increase GTPase activity of G-proteins by binding to the C-terminus of G_i/G_o α-subunit, mimicking in this

way the action of G-protein coupled receptors [1]. However, it has not yet been clarified which structural elements of amphipathic peptides are crucial for their activity. In order to shed some light on this problem we have tested eight different amphipathic peptides as potential modulators of GTPase activity of G-proteins and correlated the obtained results with the peptide sequences, their predicted secondary structures and calculated solvent accessibility. The following amphipathic peptides were studied: mastoparan, MAS17 (inactive mastoparan analog) [1], melittin (a component of bee venom) [2], M252 and M256 (sequences (272-291) and (370-383) from the intracellular loop of p75 nerve growth factor receptor) [3], PD_1 (synthetic peptide detergent-peptitergent) [4], M366 (β-amyloid protein (25-35)) [5], and cys-pAntp (*Drosophila* homeoprotein Antennapedia (43-58)) [6] .

METHODS

Peptide synthesis: Peptides were synthesised by solid phase synthesis using *t*-Boc-chemistry . Peptides were synthesised in a stepwise manner on a 0.1 mmol scale using an Applied Biosystem Model 431A peptide synthesiser as described earlier [7].

Plasma membranes preparation: Wistar rats were sacrificed, brain was removed and sliced, brain cortex separated and quickly frozen in liquid nitrogen. Membranes were prepared according to the protocol of McKenzie [8], with minor modifications. The protein concentration in the obtained preparation was determined by the method of Lowry [9]. Membranes were then diluted in TRIS-EDTA buffer pH 7.5 and were used in the final protein concentration of 2.21 mg/ml.

GTPase assay: The determination of GTPase enzymatic activity was performed radiometrically according to Cassel and Selinger [10], with the modifications suggested by McKenzie [8]. The total concentration of GTP was 0.5 μM with trace amounts of $\gamma[^{32}P]$GTP to give 50.000 - 100.000 cpm in an aliquot of the reaction cocktail in which plasma membranes diluted in Tris-EDTA buffer pH 7.5 were added. Background low-affinity hydrolysis of $\gamma[^{32}P]$GTP was assessed by incubating parallel tubes in the presence of 100 μM GTP. Blank values were determined by the replacement of rat brain cortex

membrane solution with assay buffer. The GTPase reaction was started by transferring the reaction mixtures to a 25°C water bath for 10 minutes. Subsequently, free [$^{32}P_i$] was separated from the unhydrolysed γ[^{32}P]GTP in 5% suspension of activated charcoal in 20 mM H_3PO_4. The amount of the yielding radioactive phosphate was determined in a LKB 1214 Rackbeta liquid scintillation counter. Basal GTPase activity of rat brain cortex plasma membranes was 0.52 pmol/min/mg protein.

Secondary structure prediction: Secondary structure prediction of the peptides and prediction of residue solvent accessibility were performed by using two PHD methods (**P**rofile fed neural network systems from **H**ei**D**elberg), PHDsec (secondary structure) and PHDacc (solvent accessibility) [11-14]. Internet accessible (http//www.embl-heidelberg.de/ predictprotein) programs installed in Heidelberg University, FRG, were used. PRISM (GraphPad Software, USA) computer program was used for the fitting of the curves and other calculations, as well as for the graphical presentation of the results.

Chemicals: [γ-^{32}P]GTP was from NEN, UK; *tert*-butyloxycarbonyl amino acids were from Chemimpex, USA; all other chemicals were from Sigma, USA.

RESULTS AND DISCUSSION

It is seen from Fig. 1 and Table 1 that four out of eight tested amphipathic peptides (mastoparan, melittin, PD_1 and M366) were able to increase GTPase activity of G-proteins. The activation with mastoparan and melittin was in accordance with a bi-phasic dose-response curve with maximally 221% and 148% of basal GTPase activity, respectively. Maximal activation of M366 and PD_1 was 211% and 194% of the basal and a single-step dose-response curve could be used. The other four peptides showed no effect within the error limits (Table 1) up to the concentration as high as 100 μM.

Comparison of the effect of the peptides on GTPase activity with the peptide sequences, predicted secondary structure and predicted residue solvent accessibility (Figs. 2 and 3) has revealed two types of GTPase activators among studied peptides. First type

represent longer peptides, PD_1 and melittin (Fig. 2), with the following features: a) complete α-helical structure in which the hydrophilic amino acids regularly alternate

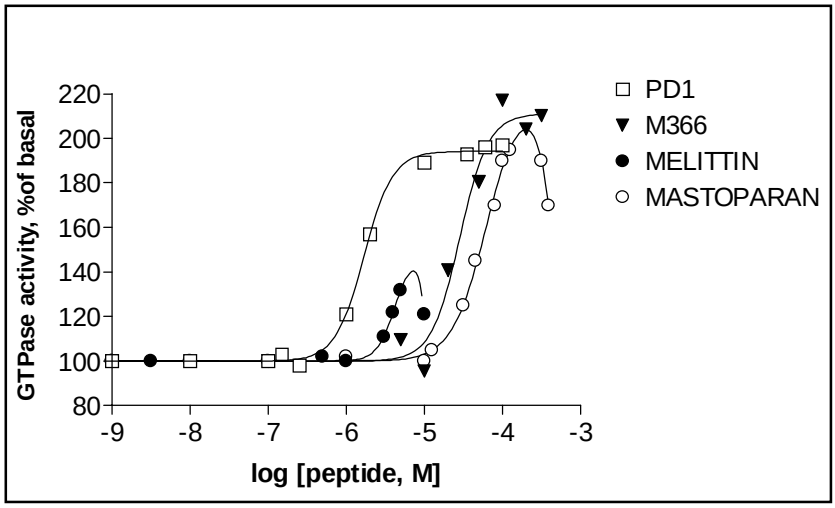

Fig. 1: Activation of GTPase by different amphipathic peptides. Standard deviation of the points was 5 - 12 % and is not shown for clarity.

Table 1: Kinetic parameters calculated from the effect of amphipathic peptides on GTPase activity of G-proteins. EC_{50} represents the concentration of the peptide with 50% effect and n_H is the Hill coefficient. For mastoparan and melittin only parameters for the ascending phase of the dose-response curves are shown.

peptide name and sequence	**max. effect** (% of basal)	**EC_{50}** (μM)	**n_H**
CONTROL **(NO PEPTIDE ADDED)**	**100 ± 10**	-	-
MASTOPARAN **I N L K A L A A L A K K I L**	**221 ± 19**	**62 ± 10**	**2.0 ± 0.3**
M366 (β-amyloid protein (25-35)) **G S N K G A I I G L M**	**211 ± 7**	**28 ± 5**	**2.4 ± 0.8**
PD_1 (peptitergent) **E E L L K Q A L Q Q A Q Q L L Q Q A Q E L A K K**	**194 ± 5**	**1.7 ± 0.2**	**2.3 ± 0.3**
MELITTIN **G I G A V L K V L T T G L P A L I S W I K R K R G G**	**148 ± 22**	**4.2 ± 2.2**	**3.8 ± 2.7**
M252 (p75 nerve growth factor receptor (272-291)) **A F K R W N S C K Q N K Q G A N S R P V**	**109 ± 8**	_	_
M256 (p75 nerve growth factor receptor (370-383)) **L D A L A A L R R I Q R A**	**107 ± 3**	_	_
MAS17 (inactive analog of mastoparan) **I N L K A K A A L A K K L L**	**102 ± 10**	_	_
cys-pAntp (*Drosophila* Anntenapedia (43-58)) **C R Q I K I W F Q N R R M K W K K**	**101 ± 10**	_	_

with hydrophobic ones, forming thus two well separated hydrophobic/hydrophilic surfaces throughout the whole length of the peptide; and b) separation of charges located at the terminal parts of the molecule. The peptides M252 and cys-pAntp (Fig. 2) are similar in alternating hydrophilic and hydrophobic amino acids, but they do not contain a complete α-helical structure and the charges are not separated within molecule; this seems to prevent them to function as GTPase activators. The results of structure prediction of PD_1 and melittin are in full accordance with the structure of these two peptides obtained by X-ray diffraction and NMR studies [2, 4]. Additional common feature of PD_1 and melittin is their strong tendency to form aggregates, mostly tetrameres [2, 4].

The second type of GTPase activators (mastoparan and M366) bears a shorter α-helix and is characterised by a specific motif consisting of positively charged lysine near N-terminus, followed by 5-6 hydrophobic amino acids (Fig. 3). In other tested short peptides which are not GTPase activators this motif is not present or is incomplete. In e. g. MAS17 an additional charged lysine occurs within the hydrophobic region (Fig. 3) and M256 includes a negatively charged residue (aspartic acid) in the vicinity of the N-terminus (Fig. 3). Both these peptides seem to be in α-helical structure (Fig. 3), but this is obviously a feature not sufficient for the activation of GTPase.

Our data are in accordance with the findings of Higashijima et al [15], who claimed that amphiphilicity of the GTPase activating peptides is of the primary importance, but the charged residues defining the amphiphilic character of the peptide may also play important role. Detert and co-workers [16] suggest that charge is not necessary for the GTPase activation, however, their results were not obtained with peptides but rather with substituted histamines, which might have different mechanism of enzyme activation. This rises also the questions such as: on which type of G-proteins the peptides act, how many interaction sites are important for the activation and where on the surface of G-proteins are these sites. It was suggested for mastoparan that it activates G-proteins by binding to C-terminal helix of G_i/G_o alpha subunit [1]. Similar structure and effect of

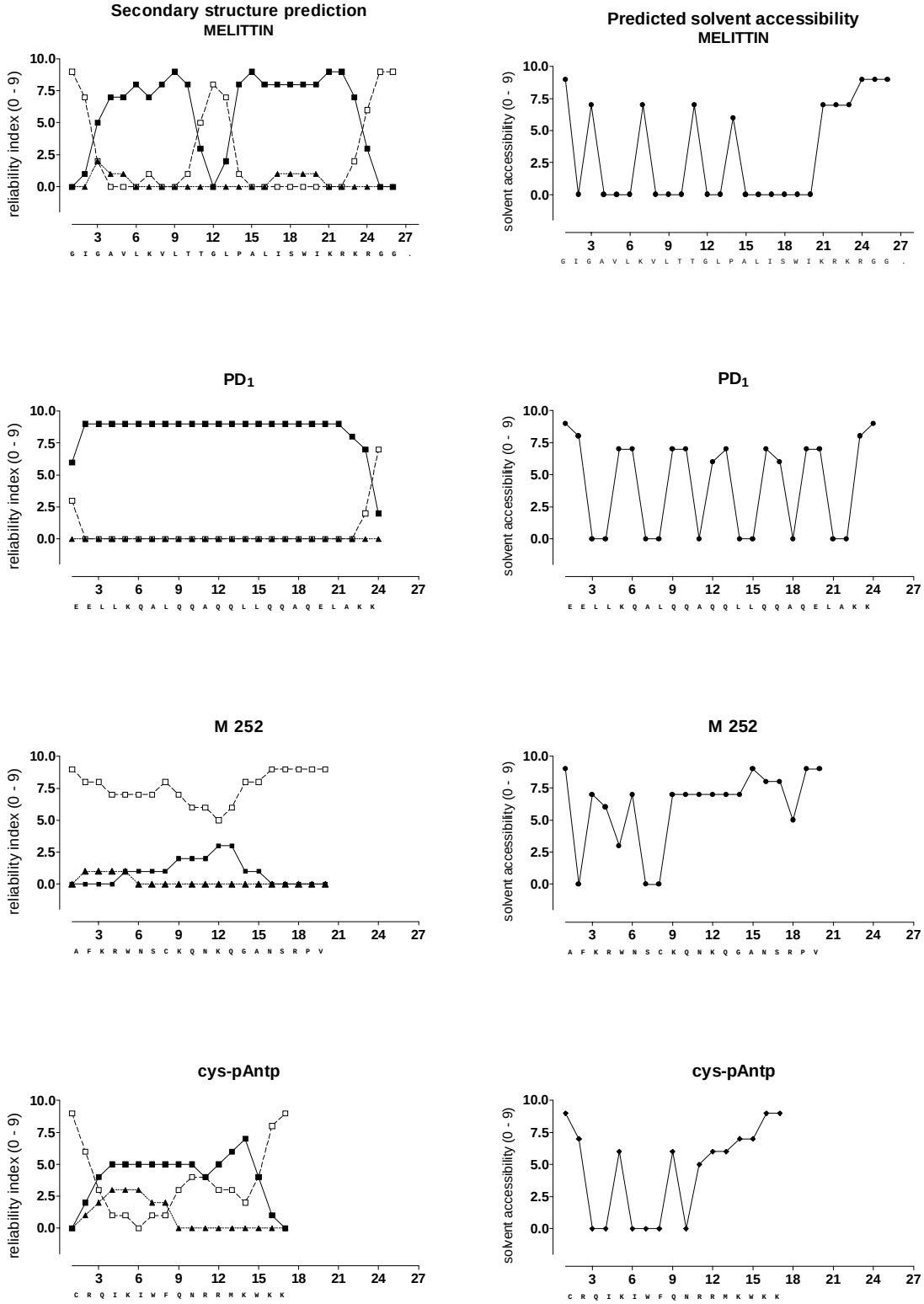

Fig. 2: Predicted secondary structure and predicted solvent accessibility of long (17-26 amino acids) amphipathic peptides. Symbols: ′, α-helix; π, β-structure; ≤, loop.

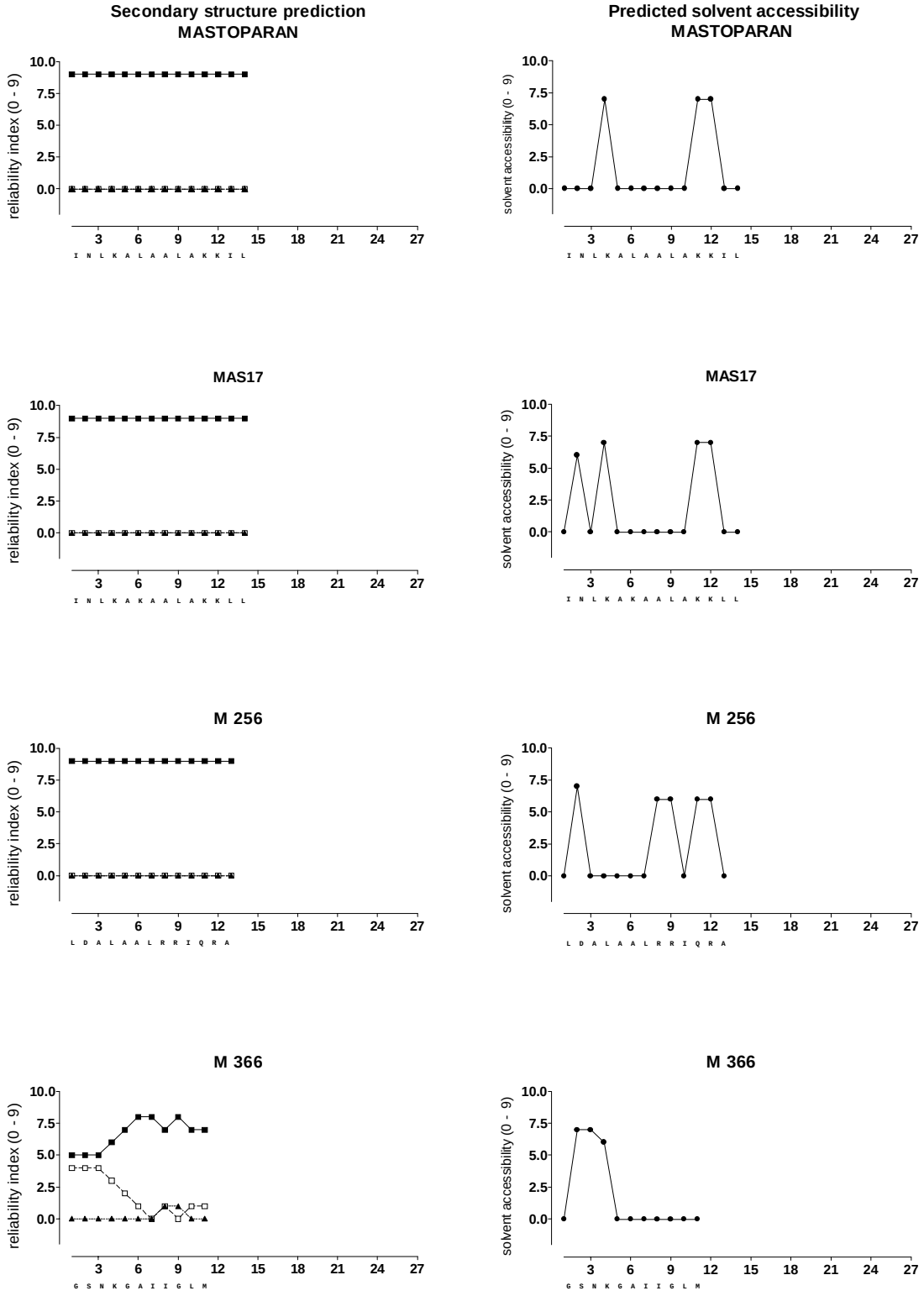

Fig. 3: Secondary structure prediction and predicted solvent accessibility of short (11-14 amino acids) amphipathic peptides. Symbols: ′, α-helix; π, β-structure; ≤, loop.

M366 and mastoparan, revealed in our study, allow us to speculate that these two peptides activate GTPase by binding to the same binding site. This is additionally corroborated by the similarity of their EC_{50} values (Table 1). Melittin and PD_1, which are structurally different from M366 and mastoparan, activate GTPase with EC_{50} which is for one order of magnitude lower than that for M366 and mastoparan. This suggests the possibility that melittin and PD_1 act on G-proteins by binding to site(s) different from that of M366 and mastoparan. Besides, the values of Hill coefficients significantly larger than 1, obtained for all GTPase activating peptides (Table 1), suggest multiple binding of the peptides to G-proteins.

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POVZETEK

Osem različnih amfipatičnih peptidov smo testirali kot modulatorje GTPazne aktivnosti G-proteinov v membranah iz korteksa podganjih možganov: mastoparan in melittin (komponenti osjega in čebeljega strupa), MAS17 (neaktivni mastoparanov analog), M252 and M256 (peptida izvedena iz receptorja za živčni rastni faktor), PD_1 (sintetični peptidni detergent-peptitergent), M366 (peptid izveden iz β-amyloidnega protein) in cys-pAntp (homeodomenski del antennapedia proteina iz *Drosophile*). Štirje peptidi (mastoparan, melittin, PD_1 in M366) so povečali aktivnost GTPaze, ostali pa niso imeli nobenega vpliva. Primerjava teh podatkov s sekvenco peptidov, njihovo napovedano sekundarno strukturo in izračunano topnostjo posameznih aminokislinskih ostankov, je pokazala na dva tipa aktivatorjev. Za prvi tip (melittin in PD_1) je značilen daljši, v celoti amfipatični a-heliks (24-26 aminokislin), z ločenimi naboji na obeh koncih peptida. Drugi tip (mastoparan in M366) predstavlja krajši a-heliks (11-14 aminokislin) in kaže značilen motiv, ki ga sestavlja lizin na mestu 4 ter 5 do 6 aminokislin z radikali, ki imajo majhno sposobnost raztapljanja v vodi.