Biochimica et Biophysica Acta, 1112 (1992) 235–240

BBAMEM 75802

Structure and activity studies of pardaxin and analogues using model membranes of phosphatidylcholine

Colin J. Barrow, Koji Nakanishi and Kazuo Tachibana

Suntory Institute for Bioorganic Research, Shimamoto-cho, Mishima-gun, Osaka (Japan)

(Received 4 June 1992)

Key words: Membrane perturbation; Pardaxin; Phosphatidylcholine; Model membrane

Thirteen synthetic pardaxin analogues were assayed for their ability to interact with model membranes of phosphatidylcholine. The results suggested the following: An amphipathic α-helix from isoleucine-14 to leucine-26 is responsible for most of the membrane perturbing properties of pardaxin. A hydrophobic N-terminal region enhances the activity of the isoleucine-14 to leucine-26 α-helix by binding the pardaxin molecule to the lipid bilayer. A bend centered around ^{12}Ser–^{13}Pro appears to create overall amphipathicity for the two different helical regions of pardaxin, but this contributes only slightly to potency. The C-terminal amino acids are unimportant for membrane perturbing activity and may be present only to enhance transportation in an aqueous environment prior to membrane binding in the native system.

Introduction

Pardaxins are a class of peptides which were isolated together with steroid glycosides as the compounds responsible for the shark repellency of defence secretions from soles of the genus *Pardachirus*. Three pardaxins (pardaxin P-1, P-2, and P-3) isolated from the pacific sole, *Pardachirus pavoninus*, were determined to be peptides, each containing 33 amino acid residues [1]. The major pardaxin from the moses sole, *Pardachirus marmoratus*, differed from pardaxin P-1 by replacement of a single residue [2].

Pardaxins exhibit a variety of biological activity including ichthyotoxicity and hemolytic activity. They also have strong surfactant properties [3]. The biological activity of the pardaxins is attributed to their ability to perturb biomembranes. In this regard pardaxins have been shown to induce aggregation, but not fusion, of phosphatidylserine vesicles [4], and to cause the release of entrapped hydrophilic molecules from phosphotidylcholine vesicles [5]. Pardaxins were also shown to interfere with ion transport in both epithelium [6] and nerve cells [7].

In unilamellar liposomes made from egg yolk phosphatidylcholine, pardaxins appear to form voltage-dependant, ion-permeable channels at concentrations below 10^{-7} M, with lysis occurring at higher concentrations [5]. Lysis occurs in unilamellar liposomes at approx. 100-times as low a concentration as that causing hemolysis to rabbit erythrocytes [8]. These observations suggest that the phospholipid bilayer is the main target of pardaxins at plasma membranes of erythrocytes and other biological cells. Although the structure of the channels have not yet been determined, recent results [9,10] suggest that a helix-bend-helix structure is the active conformation of pardaxin.

Although the properties of the pardaxins are similar to those of other membrane interactive peptides such as melittin [11], gramicidin [12] and alamethicin [13], the primary structures, net charge and lengths of these peptides are quite different. We have therefore tried to evaluate the influence of these factors on the ability of pardaxins to interact with membranes. We describe here the action of various synthetic pardaxin analogues on unilamellar vesicles of phosphatidylcholine as an aid to elucidating which structural features are important for membrane perturbation.

Experimental procedures

Peptide synthesis and purification. All peptides were synthesized on solid-phase in an automated fashion using a peptide synthesizer (Applied Biosystems model 430A) with the ready made standard microcomputer software, using the conventional *tert*-butyloxycarbonyl (t-Boc) strategy. The peptides were synthesized starting with 0.5 mmol of the C-terminal amino acid bonded to

Correspondence to: C.J. Barrow, Sterling Winthrop Pharmaceuticals, Research Division, 25 Great Valley Parkway, Malvern, PA 19355, USA.

TABLE I

Amino acid sequence of pardaxin and analogues

Peptide No.	Peptide designation	Sequence			
1	Pardaxin P-1	GFFALIP[7]	KIISSP[13]	LFKTLLSAVG[23]	SALSSSGEQE
2	Des[24–33]pardaxin	GFFALIP	KIISSP	LFKTLLSAVG	----------
3	Des[1–4]pardaxin	----LIP	KIISSP	LFKTLLSAVG	SALSSSGEQE
4	Des[14–33]pardaxin	GFFALIP	KIISSP	----------	----------
5	Fmoc-pardaxin	Fmoc-GFFALIP	KIISSP	LFKTLLSAVG	SALSSSGEQE
6	Acetyl-pardaxin	Ac-GFFALIP	KIISSP	LFKTLLSAVG	SALSSSGEQE
7	[Ser[8]]pardaxin	GFFALIP	SIISSP	LFKTLLSAVG	SALSSSGEQE
8	Des[14–23]pardaxin	GFFALIP	KIISSP	----------	SALSSSGEQE
9	Des[8–13,24–33]pardaxin	GFFALIP	--------	LFKTLLSAVG	----------
10	Des[8–13]pardaxin	GFFALIP	--------	LFKTLLSAVG	SALSSSGEQE
11	Des[Pro[13]]pardaxin	GFFALIP	KIISS	LFKTLLSAVG	SALSSSGEQE
12	[Leu[13]]pardaxin	GFFALIP	KIISSL	LFKTLLSAVG	SALSSSGEQE
13	[K[9]LLE]pardaxin	GFFALIP	KKLLESP	LFKTLLSAVG	SALSSSGEQE
14	[S[9]SSSS]pardaxin	GFFALIP	KSSSSSSP	LFKTLLSAVG	SALSSSGEQE

phenylacetamidomethyl (PAM)-resin to give the resin-linked protected peptides. The free peptides were obtained by deresination and simultaneous deprotection with anhydrous hydrogen fluoride (HF). These peptides and their amino acid sequences are listed in Table I.

All peptides were purified using reverse phase high pressure liquid chromatography (rpHPLC) using gradients of acetonitrile in 0.05% aqueous trifluoroacetic acid (TFA) on a Cosmosil $5C_4$-300 column (Nacalai tesque, Osaka). The synthetic samples to be purified were injected as solutions in either neat dimethylsulfoxide or 27% aqueous n-propanol. The homogeneity of each purified peptide was confirmed by rpHPLC using a Vydac Protein C4 column and a LiChrosorb RP-18 (7 μm) column and by quantitative amino acid analysis on a Tosoh CCP & 8000 amino acid analyzing system (Table II). Sequences were confirmed by sequence analysis on a Shimadzu PSQ-1 Protein Sequencer. Mass spectra were obtained for the N-terminal derivatized peptides on a ZAB SEQ mass spectrometer, using fast atom-bombardment (FAB) ionization.

Pardaxin with acetylated N-terminal (peptide 6). Resin-linked protected Pardaxin P-1 (150 mg) was stirred for 30 min in 50% TFA in dichloromethane (4 ml). The solvent was removed and the resin-linked peptide was washed with ether (2 ×) followed by 10% diisopropylethylamine (DIEA) in diethyl ether (2 ×) and finally dimethylformamide (DMF). Then acetic anhydride (500 μl) in DMF (2 ml) was added and the mixture stirred for 20 min. After washing with DMF (3 ×) and diethyl ether (3 ×) the resin-linked peptide was deprotected and purified using rpHPLC as before, to give monoacetylated pardaxin P-1 (peptide 6); fabms *m/z* 3436.41 $[M + H]^+$ ($C_{159}H_{254}N_{36}O_{48}$ requires 3436.88).

Pardaxin P-1 with 9-fluorenylmethyloxycarbonyl. (Fmoc) derivatized N-terminal (peptide 5). Resin-linked protected pardaxin P-1 (150 mg) was stirred for 30 min in 50% TFA in dichloromethane (4 ml). The

TABLE II

Amino acid analysis of pardaxin and analogues [a]

Peptide	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Phe	Lys
1	0.8(1)	6.2 (7)	3.3(3)	1.8(2)	3.4(3)	3.0(3)	1.0(1)	2.9(3)	4.8(5)	2.5(3)	1.5(2)
4		2.1 (2)		1.8(2)	1.0(1)	1.2(1)		2.6(3)	1.3(1)	2.2(2)	1.0(1)
7	0.9(1)	7.2 (8)	3.3(3)	1.9(2)	3.5(3)	3.0(3)	1.0(1)	3.0(3)	5.0(5)	2.6(3)	0.9(1)
8		5.1 (6)	3.4(3)	1.8(2)	2.4(2)	2.0(2)		2.9(3)	2.0(2)	1.7(2)	0.9(1)
9	0.9(1)	0.8 (1)		0.9(1)	2.4(2)	2.0(2)	1.0(1)	0.9(1)	3.8(4)	2.6(3)	0.9(1)
11	0.8(1)	6.4 (7)	3.3(3)	1.0(1)	3.4(3)	3.0(3)	1.0(1)	2.9(3)	5.0(5)	2.6(3)	1.6(2)
12	0.9(1)	7.3 (7)	3.5(3)	0.8(1)	3.2(3)	3.1(3)	1.0(1)	2.6(3)	6.4(6)	3.0(3)	2.2(2)
13	0.8(1)	6.3 (6)	4.5(4)	1.6(2)	3.1(3)	3.2(3)	1.0(1)	1.0(1)	7.5(7)	3.1(3)	3.2(3)
14	0.9(1)	11.1(11)	3.4(3)	1.7(2)	3.2(3)	3.0(3)	1.0(1)	0.9(1)	5.5(5)	3.1(3)	2.1(2)

[a] Structural analysis for the natural product, peptide 3, and the synthetic compounds, peptides 2 and 10, were reported previously [1,8].

solvent was removed and the resin-linked peptide was washed with diethyl ether (2 ×) followed by 10% DIEA in diethyl ether (2 ×) and finally DMF. An ice-cooled solution of Fmoc-Cl (20 mg) in dioxane (0.4 ml), water (0.07 ml) and triethylamine (7 μl) was then added to the resin. Basicity was monitored with pH paper and the mixture was stirred for 2 h at room temperature. After washing with DMF (3 ×) and diethyl ether (3 ×) the resin-linked peptide was deprotected and purified as before to give N-terminal Fmoc derivatized pardaxin P-1 (peptide 5); fabms *m/z* 3653.41 $[M + Na]^+$ ($C_{173}H_{252}N_{36}O_{47}Na$ requires 3653.11).

Interaction of peptides with lipid vesicles. The following procedure was employed based on a method reported by Kanellis et al. [14]. A mixture of 5- and 6-carboxyfluorescein (1 mmol) was suspended in a 4 ml solution of 2 mM *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyltaurine and 2 mM histidine (Tes-His buffer). The suspension was brought to pH 7.4 with approximately 1 ml of 2 M aqueous sodium hydroxide, leading to the dissolution of the acidic dye. Egg yolk lecithin (Sigma) was suspended in this 0.2 M dye solution (2 ml) with vortex mixing and ultrasonicated at 0°C under anaerobic atmosphere at 50 W power discontinuously for 30 min. The mixture was then centrifuged at 1000 × *g* for 5 min and unilamellar liposomes were fractionated from the resultant supernatant using gel filtration through Sepharose 4B eluted with the Tes-His buffer containing 0.15 M sodium chloride. This solution was then diluted with the same saline buffer to a desired phospholipid content based on an enzymocolorimetric quantification of the mother solution. The sample solution of a known concentration in 50% aqueous trifluoroethanol (TFE) (50 μl or below) was added to the dilute liposome solution (2.5 ml) with immediate stirring, and the increase in fluorescence from the solution was monitored at 25°C with excitation at 490 nm and emission at 517 nm. 10% aqueous Triton X-100 (20 μl) was added to the solution later to obtain the fluorescence increase equivalent to 100% leakage. This was repeated at several peptide concentrations for each sample. Addition of the solvent alone elicited no leakage. EC_{50} values, referring to the concentration required to elicit 50% dye leakage after 2 min from the time of peptide addition, were intrapolated from the obtained results (Table III).

Circular dichroic (CD) spectroscopy. CD spectra were recorded on a Jasco J-600 spectropolarimeter in cells of light path length 0.5 cm. The concentration of the peptide stock solutions was determined by quantitative amino acid analysis. Results were obtained from samples using three different solvent ratios (3:1, 1:1, 1:3 water/TFE) and two different pH levels (3.5 and 7.2) with 5 mM sodium phosphate buffer. Percent α-helix, β-sheet and random coil were calculated based on the method of Greenfield and Fasman [15] and are given in Table IV for the case of 1:1 water/TFE at pH 7.2.

Results and Discussion

Initially, we examined the secondary structure of pardaxin P-1 in order to decide which derivatives to synthesize and assay. The structure of pardaxin P-1 was initially investigated by applying the predictive method of Greenfield and Fasman [15] in conjunction with circular dichroism (Table IV). This investigation suggested that the peptide forms a similar ordered structure in the presence of sodium dodecyl sulfate micelles, presumably a membrane-interacting environment, and in aqueous trifluoroethanol [16]. It consequently led us to determine the solution structure of pardaxin P-2 in aqueous trifluoroethanol, using 1H nuclear magnetic resonance (NMR) spectroscopy and distance restrained molecular dynamics [9]. One- and two-dimensional NMR spectra of pardaxin P-1 were almost identical with those obtained for pardaxin P-2 implying that the solution structures of these peptides are very similar (unpublished data). Indistinguishable bioassay results, CD spectra, and chromatographic behavior for pardaxin P-1 and P-2 [1] adds further support for very similar secondary structures for these peptides. The above NMR investigation indicated that pardaxins are composed of four distinct regions: ^{1}Gly–^{7}Pro, ^{8}Lys–^{13}Pro, ^{14}Ile–^{26}Leu and ^{27}Ser–^{33}Glu. Both of the terminal segments exist in flexible conformations while the ^{8}Lys–^{13}Pro region has helical character and is amphipathic. The ^{14}Ile–^{26}Leu region is an amphipathic α-helix as predicted previously [16]. The hydrophobic and hydrophilic sides of the ^{8}Lys–^{13}Pro and ^{14}Ile–^{26}Leu regions are aligned with the aid of a bend of approximately 80° centered around ^{12}Ser–^{13}Pro, so that the entire ^{8}Lys–^{26}Leu region forms a boomerang-shaped amphipathic configuration [9]. The synthetic peptides below are analogues of pardaxin P-1, which is the major pardaxin isolated from *P. pavoninus*. The pardaxin analogues (Table I) were chosen to allow an assessment of the importance for membrane perturbation of each distinct structural region within pardaxin. We also investigated the contributions of the bend centered around proline-13, and the charges on lysine-8 and the N-terminal, to membrane perturbation.

N-Terminal amino acids and charge

We began by investigating the importance of the hydrophobic N-terminal amino acids and also the N-terminal charge on the ability of pardaxins to perturb phospholipid bilayers. Pardaxin P-1 and P-2 minus the first four amino acids were previously shown to retain the strong surfactancy of the pardaxins, but to be neither ichthyotoxic nor hemolytic [1]. Also, liposomes are approximately 30-times less sensitive to perturba-

TABLE III

Ability of pardaxin and its analogues to release carboxyfluorescein from unilamellar vesicles [a]

Peptide No. [b]	EC_{50} [c] (nM)
1	13 (35) [d]
2	(48)
3	(1100)
4	16000
5	17
6	25
7	18
8	> 80000
9	80
10	(130)
11	90
12	10
13	100
14	4000

[a] Phospholipid concentration was $3.95 \cdot 10^{-5}$ M.

[b] Peptide numbering is according to Table I.

[c] EC_{50} (50% fluorescence after 2 min) values are given in nM and were obtained for each sample using curve fitting of three to six data points, with at least one point each between 30 and 50% and between 50 and 70% leakage. Maximum error is ±50% for EC_{50} values.

[d] The bracketed values were obtained in an earlier assay run with a different phospholipid concentration [8].

tion by the shorter peptide (peptide 3) than to the complete pardaxins [8] (Table III).

To investigate whether the N-terminal amino acids are themselves responsible for pardaxin activity or merely necessary for it, we synthesized the N-terminal fragment ^{1}Gly–^{13}Pro (Table I, peptide 4). This peptide showed approximately a 1000-fold decrease in activity relative to that observed for pardaxin, using our liposomes assay. This result shows that although the N-terminal is essential to pardaxins ability to interact with phospholipid bilayers, this region is not the active region of the pardaxin molecule. The most probable explanation for the necessity of this region for activity is that it anchors pardaxin onto the membrane by acting as a hydrophobic binder.

To investigate the importance of the N-terminal charge in the possible membrane binding role of this region, we synthesized pardaxin P-1 acetylated at the N-terminal (peptide 6) and also pardaxin P-1 with the large hydrophobic Fmoc group at the N-terminal (peptide 5). The structures of these derivatives were confirmed by mass spectrometry after purification using rpHPLC. The activities of both these compounds were similar to that of pardaxin P-1 (Table III), implying that the N-terminal charge is not of major importance in membrane binding, at least in the case of phosphatidylcholine vesicles where the overall surface charge is neutral. The N-terminal does not, therefore, interact strongly with the ionic surface of the bilayer. The binding may instead be due to hydrophobic interactions where initial contact between pardaxin and the lipid bilayer occurs via contact of the hydrophobic N-terminal amino acids with the lipid chains. Once the hydrophobic N-terminus is anchored into the bilayer, the remaining peptide segments should be sufficiently close to the membrane for helix formation to occur (in high dilution in water, pardaxin is essentially random coil, as seen by CD and NMR spectroscopy). Because the N-terminal charge is not important for activity, the mechanism of anchoring is probably one where the N-terminal is buried within the hydrophobic core of the membrane. This is also supported by a recent result where pardaxin, derivatized to form an acidically charged N-terminal appeared to insert more slowly into liposomes [10].

C-Terminal amino acids, central amphipathic α-helix, and overall length

Pardaxin without the C-terminal amino acids (peptide 2) has previously been shown to exhibit similar activity to that shown by pardaxin [8] (Table III). This suggests that the hydrophilic C-terminal is not involved in membrane perturbation and may be present only to enhance the solubility in seawater prior to binding to the membrane in the native system. In contrast, peptide 8 (Table I), which is equivalent to pardaxin with deletion of the main amphipathic α-helical region, was completely inactive in our assay. These results show that the central helical region is essential to the membrane perturbing ability of the pardaxins and is probably the main membrane disruptive region in these peptides.

To investigate the effect of peptide length on the liposome interactive ability of the pardaxins and also to further establish the importance of the N-terminal amino acids and the ^{14}Ile–^{26}Leu helix for activity, we synthesized a pardaxin analogue 17 amino acids in length, containing only these two regions (Table I, peptide 9). It is interesting to note that the end on end lengths of pardaxin and melittin are almost identical. [9], which led us to speculate that length may play an important role in the activity of these peptides. However, the activity of peptide 9 was only about six times lower than that of pardaxin (Table III). Because this peptide is probably too short to span the lipid bilayer this result suggests that pardaxin does not have to span the bilayer membrane for activity, and that overall length is not an important factor in the ability of pardaxin to permeabilize lipid bilayers. The activity of peptide 9 again supports the importance of the N-terminal amino acids and central helical region for pardaxins membrane perturbing ability.

The ^{8}Lys–^{13}Pro region, including the lysine-8 charge and the bend region

The six times lower activity of peptide 9, as compared to pardaxin or peptide 7, and the unusual bend centered at proline-13, led us to investigate the role of this small amphipathic partially helical region in pardaxins activity. First, we decided to investigate the potential role of the charge at lysine-8 in stabilizing the membrane peptide interaction, possibly through assisting in binding via interaction with the charged membrane surface. To do this we synthesized a pardaxin analogue with lysine-8 replaced with serine (peptide 7). However, our assay gave no significant difference in activity from that of pardaxin (Table III). The positive charge here therefore appears to have no significant role in the interaction of pardaxin, at least with liposomes made from phosphatidylcholine. This, however, does not eliminate the possibility that this, and other charges, plays a role in the ion selectivity of the pardaxin channel at lower concentrations [17].

If this ^{8}Lys–^{13}Pro region were just a spacer region to separate binding and active regions, it should be possible to replace it with a sequence of uninteractive amino acids without loss of activity. To test this hypothesis an analogue was synthesized with K^{8}SSSSSSP replacing K^{8}IISSP (peptide 14). Serine was chosen so that the bend would be retained, but the amphipathicity lost in this region. The activity of this analogue in the liposome assay was approximately 400 times lower than that of pardaxin (Table III). Therefore this region is not merely a spacer but has some necessary structural elements that contribute to the ability of pardaxin to perturb membranes. We suggest that a certain hydrophobicity is necessary for this region and that a long serine region inhibits deep insertion of the active amphipathic α-helical into the membrane.

NMR spectroscopic analysis of pardaxin showed that the ^{8}Lys–^{13}Pro region has a bend of approx. 80° centered around ^{12}Ser–^{13}Pro [9]. Structurally the effect of this bend is to allow both amphipathic helical segments to present their polar residues on one common face [9]. We hypothesized that an overall amphipathicity may be necessary for pardaxins membrane perturbing ability. The importance of this bend, and overall molecular amphipathicity, was investigated by the synthesis of a pardaxin analogue with deletion of proline-13 (peptide 11) and also its replacement by leucine (peptide 12). Circular dicroism of these peptides supported increased helical content for both peptides, as compared to that of pardaxin (Table IV), suggesting the formation of an extended helical region. Peptide 11 should retain overall helical amphipathicity while it should be twisted in peptide 12. The activity of peptide 11 in the liposome assay was 6-fold lower than that of pardaxin showing that the bend is an important feature in this region and has some importance to the ability of pardaxin to perturb lipid bilayers, but is not a crucial element for activity. However, the activity of peptide 12 was indistinguishable from that of pardaxin (Table III), despite the twisted pathicity dipole. A possible explanation for the equivalent activity of these two peptides is that once peptide 12 is placed in an environment like the membrane surface, it adopts the more energetically favorable bent shape similar to pardaxin, becoming completely amphipathic. Another explanation is that combined amphipathicity between the two helical regions of pardaxin is not important for activity.

TABLE IV

Distribution of secondary structure for pardaxin and analogues estimated from circular dichroic spectra [a,b]

Peptide No.	% helix	% beta sheet (±10%)	% random coil (±10%)
1	40	20	40
4	15	10	75
7	55	5	40
8	5	20	75
9	45	25	30
11	55	10	35
12	55	10	35
13	55	5	40
14	40	20	40

[a] Spectra were measured for peptide solutions in 1:1 water/trifluoroethanol with potassium phosphate buffer (5 mM) at pH 7.2.

[b] Percentages of secondary structure were obtained according to the method of Greenfield and Fasman [15].

To investigate whether we could make a more active analogue by increasing the amphipathic helicity of the ^{8}Lys–^{13}Pro region, we replaced K^{8}IISSP with K^{8}KLLESP (peptide 13). This should produce a pardaxin analogue with two definite helical regions separated by a bend, as is the case for melittin [18], and also retain overall molecular amphipathicity. This sequence was chosen because leucine has a high helix forming tendency [19] and lysine-8 should form a salt bridge with the glutamic acid three residues away stabilizing helix formation in this region [20]. Increased helical content for peptide 13, compared with pardaxin, was confirmed using CD (Table II). However, assay data showed a lower activity for this peptide (Table III). This shows that a second helical region between the N-terminus and the main helix does not necessarily increase pardaxin membrane perturbing ability.

Although the ^{8}Lys–^{13}Pro region is not itself very important for pardaxin activity toward liposomes, it cannot be replaced by a combination of any random series of amino acids without loss of activity. Although the role of this region is not completely clear, it is probably necessary that some hydrophobicity together with some rigidity exists in this region so that the N-terminal residues do not interact unfavorably with

the main active amphipathic α-helical region of pardaxin, and/or that the spatial alignment between the two regions is favorably maintained.

Pardaxins' ability to form membrane pores and to cause lysis of phopholipid bilayers is dependant on several structural features of the pardaxin molecule. The most important features appear to be the presence of a hydophobic N-terminal and an amphipathic α-helix. In aqueous solution below a certain concentration, pardaxins have been shown by CD spectroscopy, to take the random coil structure [16]. Because presence of the hydrophobic N-terminal amino acid residues are desirable for pardaxins to perturb liposomes, it appears to be this region, probably the phenylanaline residues at position 2 and 3 in particular, which provide an initial hydrophobic contact with the lipid bilayer. Once the hydrophobic N-terminus embeds in the hydrophobic core of the bilayer, the remaining amino acids are close enough to contact the bilayer with spontaneous formation of an amphipathic α-helix over the ^{14}Ile-^{26}Leu region. At this stage, it is probably energetically favorable for the residues from lysine-8 to proline-13 to form an amphipathic partial helix so that the overall molecule is amphipathic. Probably the C-terminal remains on the outer surface of the bilayer, and may have some role in ion selectivity in the case of channel formation.

Acknowledgments

We thank H. Naoki and P.T.M. Kenny for mass spectroscopic analysis, W. Miki and Y. Kamatani for some amino acid analysis and peptide sequencing and S.A. Thompson for helpful discussions.

References

1 Thompson, S.A., Tachibana, K., Nakanishi, K. and Kubota, I. (1986) Science 233, 341–343.
2 Thompson, S.A., Tachibana, K., Kubota, I. and Zlotkin, E. (1988) Peptide Chemistry 1987, 127–132.
3 Primor, N. and Zlotkin, E. (1975) Taxicon 13, 227.
4 Lelkes, P.I. and Lazarovici, P. (1988) FEBS Lett. 230, 131–136.
5 Lazarovici, P., Primor, N. and Loew, L.M. (1986) J. Biol. Chem. 261, 16704–16713.
6 Primor, N. (1983) J. Exp. Biol. 105, 83–94.
7 Renner, P., Caratsch, C.G., Waser, P.G., Lazarovici, P. and Primor, N. (1987) Neuroscience 23, 319–325.
8 Tachibana, K., Xu, W.-H., Barrow, C.J. and Imoto, S. (1989) Peptide Chemistry 1988, 279–284.
9 Zargorski, M.G., Norman, D.G., Barrow, C.J., Iwashita, T., Tachibana, K. and Patel D.J. (1991) Biochemistry 30, 8009–8017.
10 Shai, Y., Bach, D. and Yanovsky, A. (1990) J. Biol. Chem. 265, 20202–20209.
11 Tosteson, M.T. and Tosteson, D. (1982) Biophys. J. 36, 109–116.
12 Andersen, O.A. (1984) Annu. Rev. Physiol. 46, 431–548.
13 Fox, Jr., R.O. and Richards, F.M. (1982) Nature 300, 531–548.
14 Kanellis, P., Romans, A.Y., Johnson, B.J., Kercret, H., Chiovetti, Jr., B., Allen, J.M. and Sergrest, J.P. (1980) J. Biol. Chem. 225, 11464–11472.
15 Greenfield, N. and Fasman, G.D. (1969) Biochemistry 8, 4108–4116.
16 Thompson, S.A., Minakata, H., Xu, W.-H., Tachibana, K., Nakanishi, K. and Kubota, I. (1987) Peptide Chemistry 1986, 181–186.
17 Loew, L.M., Benson, L., Lazarovici, P. and Rosenberg, I. (1985), Biochemistry 24, 2101–2104.
18 Inagaki, F., Shimada, I., Kaqaguchi, K., Hirano, M., Terasawa, I., Ikura, T. and Go, N. (1989) Biochemistry 28, 5985–5991.
19 Padmanabhan, S., Marqusee, S., Ridgeway, T., Laue, T.M., and Baldwin, R.L. (1990) Nature 344, 268–270.
20 Marqusee, S. and Baldwin, R.L. (1987) Proc. Natl. Acad. Sci. USA 84, 8898–8902.