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Simultaneous solubilization of high-affinity receptors for VIP and glucagon and of a low-affinity binding protein for VIP, shown to be identical to calmodulin

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Anion-exchange chromatography of solubilized pig liver cell membranes on DEAE-Sepharose gave a fraction with high affinity binding proteins for VIP and glucagon distinct from each other. Scatchard analysis indicated the presence of one binding site for VIP (K_d 1.5 ± 0.6 nM and B_{max} 1.3 ± 0.4 pmol/mg). The order of potency for VIP-related peptides to inhibit [^{125}I]VIP binding was: VIP > peptide histidine isoleucine amide (PHI) > rat growth hormone releasing factor (rGRF) > secretin. GTP-γ-S inhibited [^{125}I]VIP binding and reduced the affinity of VIP binding sites to 6.5 nM. In the same isolated fraction, [^{125}I]glucagon binding was displaced by glucagon preferentially to oxyntomodulin, and GTP did not affect this [^{125}I]glucagon binding. Scatchard analysis indicated the presence of one binding site for glucagon (K_d 0.08 ± 0.03 nM and B_{max} 0.31 ± 0.01 pmol/mg). A low-affinity VIP binding protein (IC_{50} 0.7 μM) was detected in a fraction eluting later and exhibited a peptide specificity: rGRF > VIP > VIP(10–28) > secretin > PHI. This rGRF-preferring protein (18 kDa) was purified and had a partial amino-acid sequence identical to that of calmodulin. Its [^{125}I]VIP binding was competitively inhibited by VIP and calmidazolium in a manner similar to that for pig brain calmodulin. Thus we have co-solubilized VIP and glucagon high affinity receptors from pig liver cell membranes and separated them from VIP-binding calmodulin.

Vasoactive intestinal polypeptide; Glucagon; Solubilization; Binding protein; Pig liver membrane

1. INTRODUCTION

Vasoactive intestinal polypeptide (VIP) is a neuropeptide that exhibits several biological activities in many tissues and organs [1]. In liver, VIP and the structurally related peptide glucagon stimulate hepatic glycogenolysis and gluconeogenesis [2]. The role of glucagon in regulating glucose metabolism has been clearly established and recent reports suggest that cell-mediated metabolism of receptor bound glucagon may be linked to hepatic glucagon processing [3]. Interestingly, C-terminal fragments of glucagon (19–29, 18–29), resulting from the interaction of glucagon and liver cells, inhibit the hepatic Ca^{2+} pump [4]. Much evidence suggests that VIP and glucagon actions depend on the presence of functional receptors [5] and liver cell membranes represent a rich source of both receptors [6,7], which can be extracted by CHAPS in an active form [8–12]. These findings suggested the possibility of a simultaneous solubilization of VIP and glucagon receptors from liver cell membranes. However, detergent treatment of VIP receptors may lead to an altered peptide specificity and lowered affinity for VIP [13,14]. Furthermore, hepatic proteases against VIP and glucagon may be present in solubilized extracts [11].

We now report the co-solubilization from pig liver cell membranes of active VIP and glucagon receptors, with retained high affinity for the ligands, peptide specificity, and separation from a low affinity VIP-binding protein apparently identical to calmodulin.

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Abbreviations: GTP-γ-S, guanosine 5′-*O*-thiotriphosphate; IC_{50}, half maximal concentration for binding inhibition; VIP, vasoactive intestinal polypeptide; PHI, peptide histidine isoleucine amide; rGRF, rat growth hormone releasing factor; GLP-2, glucagon-like peptide-2; GLP-1, glucagon-like peptide-1

2. MATERIALS AND METHODS

2.1. *Materials*

(3-[^{125}I]tyrosyl^{10})VIP and (3-[^{125}I]tyrosyl^{10})glucagon were obtained from Amersham and are referred to as [^{125}I]VIP and [^{125}I]glucagon, glucagon was obtained from Novo (Bagsvaerd, Denmark), synthetic rat growth hormone releasing factor (rGRF), human glucagon-like peptide-2 (GLP-2), human glucagon-like peptide-1 (GLP-1(7–36)) amide and VIP(10–28) from Peninsula (Merseyside, UK). CHAPS, leupeptin, pepstatin and GTP-γ-S, pig brain calmodulin and calmidazolium from Boehringer (Mannheim, Germany), bacitracin and bovine serum albumin from Sigma, disuccinimidyl suberate from Pierce (Rockford, IL, USA), and wheat germ agglutinin from IBF (Villeneuve-la-Garenne, France). Oxyntomodulin was a gift from Dr. D. Bataille, CCIPE, Montpellier, France. VIP, peptide histidine

isoleucine amide (PHI) and secretin were isolated from pig upper intestine, as described [15–17].

2.2. *Preparation of pig liver cell membranes*

Crude pig liver membranes were prepared according to [18] with a homogenizing buffer of 10 mM triethanolamine (pH 7.8), 0.25 M sucrose, 5 mM $MgCl_2$, 2.5 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride. The final pellet was washed twice with 5 mM HEPES-KOH (pH 8.0) and 25 mM Tris-HCl (pH 7.5), respectively, and kept at −80°C until use. Protein concentration was determined with the BCA reagent from Pierce (Rockford, IL, USA) and bovine serum albumin was used as standard.

2.3. *Solubilization of membrane proteins*

Pig liver cell membranes were suspended (5 mg protein/ml; 25 mM Tris-HCl, pH 7.7, with 0.4% CHAPS, 25% glycerol, 5 mM $MgCl_2$ and 0.1 mM phenylmethylsulfonyl fluoride) and stirred for 20 min at 4°C. After centrifugation (100,000 × *g* for 30 min) the supernatant was filtered through 0.45 μm Millipore filters and stored at −80°C.

2.4. *Binding studies*

CHAPS solubilized extracts (50–250 μl) were incubated with [^{125}I]VIP (20–50 pM) in the presence or absence of unlabeled VIP in 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 0.3% bovine serum albumin and 0.01% bacitracin in a final volume of 400 μl. After 30 min at 20°C, receptor-bound [^{125}I]VIP was separated from free ligand by addition of 1.5 ml ice-cold dextran-coated charcoal (0.5% Norit A and 0.05% Dextran T-70), followed by centrifugation at 2,500 × *g* for 5 min. The radioactivity present in the collected supernatant was counted in a gamma-counter. The binding of [^{125}I]glucagon (20–50 pM) to CHAPS solubilized extracts (50–200 μl) in the presence or absence of unlabeled glucagon was measured at 4°C for 12 h, final volume 400 μl, assay buffer 50 mM Tris-HCl (pH 7.5), 0.1% bovine serum albumin, 0.01% bacitracin, 0.1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin. The [^{125}I]glucagon–receptor complex was separated from free ligand using dextran-coated charcoal. The non-specific binding measured in the presence of 1 μM unlabeled VIP or glucagon was subtracted to obtain specific binding and corresponded to about 10% of the total radioactivity.

2.5. *DEAE chromatography of CHAPS-solubilized membrane proteins*

The solubilized liver membrane extract (1,000 ml) was applied to a column (5 × 20 cm) of DEAE-Sepharose Cl-6B, equilibrated at 4°C with 20 mM Tris-HCl (pH 8.0), 40 mM NaCl, 20% glycerol and 0.1% CHAPS, at a flow rate of 130 ml/h. Adsorbed proteins were eluted with a gradient of NaCl in the equilibrating buffer. Aliquots of 100 μl from 12 ml fractions were assayed for [^{125}I]VIP and [^{125}I]glucagon binding. Fractions containing VIP- and glucagon-binding activities were termed A, B, C and D according to their elution order and stored at −80°C.

2.6. *Wheat germ agglutinin-affinity chromatography*

Two ml of agarose-bound wheat germ agglutinin in a chromatographic tube (1.1 × 10 cm) was equilibrated in 50 mM Tris-HCl (pH 7.5), 20% glycerol and 0.1% CHAPS. Two-ml samples were mixed with the gel for 12 h at 4°C. After washing with 5 vols. equilibration buffer, adsorbed glycoproteins were eluted with 5 vols. of the buffer containing 0.3 M *N*-acetyl-D-glucosamine. Aliquots (250 μl from fractions of 2 ml) were assayed for [^{125}I]VIP and [^{125}I]glucagon binding activities.

2.7. *Cross linking and SDS-PAGE analysis*

Cross-linking studies were performed with DEAE-purified fraction D (Fig. 1). [^{125}I]VIP (0.1 nM) was incubated with 100 μl of fraction D in the presence or absence of unlabeled VIP under the binding conditions described above. Samples were cross-linked with disuccinimidyl suberate (25 mM in dimethyl sulfoxide), which was added at a final concentration of 0.5 mM. After 15 min on ice, the reaction was quenched with 30 mM glycine.

A fivefold concentrated sample buffer was added to cross-linked incubates to obtain a final sample buffer of 50 mM Tris-HCl (pH 6.8), 10% glycerol and 2% (w/v) SDS. The samples were heated at 60°C for 20 min before SDS-PAGE analysis [19] with a 4% acrylamide stacking gel and 10–15% acrylamide resolving gel. Stained and destained gels were dried and exposed to Fuji X-ray film with an intensifying screen (Kyokko BX-III) at −80°C for 5–10 days.

2.8. *Purification of a low affinity binding protein for VIP*

Fraction D (120 ml) was concentrated in a Diaflo cell (cut-off filter 10,000 MW, Millipore) to 10 ml and applied to Sephacryl S-100 HR (2.6 × 63 cm), equilibrated in 25 mM Tris-HCl (pH 7.6), 25 mM NaCl and 0.1% CHAPS. Fractions of 5.5 ml were collected at a flow rate of 130 ml/h. Aliquots of 100 μl were assayed for specific binding. The active fractions were pooled and incubated with 5 ml VIP-affinity gel, prepared by coupling 0.4 μmol VIP per ml CNBr-activated agarose, according to the manufacturer's protocol (Pharmacia, Sweden), and equilibrated in 50 mM Tris-HCl (pH 7.5), 0.1% CHAPS. After 5 h of incubation at 4°C, the gel was poured into a chromatographic tube (1 × 7 cm) and washed with 5 vols. buffer. Bound proteins were eluted with buffer containing 0.2 M NaCl and fractions with [^{125}I]VIP binding activity concentrated using Centricon-10 microconcentrators to a final volume of 0.5 ml. This sample was diluted with 0.5 ml 20 mM Tris-HCl (pH 7.0), 0.1% CHAPS and chromatographed on Mono Q 5/5 at a flow of 1 ml/min. The [^{125}I]VIP binding fraction was eluted with increasing concentrations of NaCl. The [^{125}I]VIP binding protein was subjected to enzymatic hydrolysis with endoproteinase Lys-C as described [20]. The fragments were separated by HPLC on reverse-phase C_{18} Vydac (4.6 × 250 mm, Hesperia, CA, USA) at a flow rate of 1 ml/min using a linear gradient of acetonitrile/0.1% trifluoroacetic acid (0–60% in 45 min). Edman degradation of the major fragment recovered was performed with an ABI 477A protein sequencer and on-line phenylthiohydantoin analysis. The protein sequence was compared against the NBRF protein data bank (release 23) using the program FASTA [21]. Binding studies were performed as described above except that 1 mM $CaCl_2$ was included into the buffer.

3. RESULTS

3.1. *Solubilization of VIP and glucagon binding sites with different CHAPS concentrations*

The optimal CHAPS concentration for solubilization of active VIP and glucagon binding sites was determined by incubation of crude pig liver membranes (5 mg/ml) at different detergent concentrations in the presence of 25% glycerol. A concomitant increase of VIP and glucagon binding to solubilized proteins was observed with a maximum binding activity at 0.4% CHAPS. At higher detergent concentrations, an inhibition of both VIP and glucagon binding was observed. An extraction yield of 60–80% was calculated by measurement of the remaining ligand binding activity of the solubilized membranes. The assay of the binding activity in the crude solubilized extract was hampered by a low ratio of specific to nonspecific binding.

3.2. *DEAE-Sepharose anion-exchange chromatography of solubilized membrane proteins*

The solubilized membrane extracts were applied to DEAE-Sepharose and the eluted fractions were assayed for VIP and glucagon binding activities. As shown in Fig. 1, several fractions (A, B, C and D) bound [^{125}I]VIP or/and [^{125}I]glucagon. The binding proteins in fraction

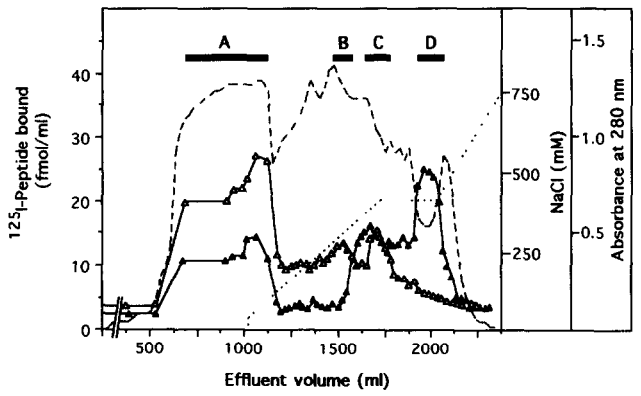

Fig. 1. DEAE-Sepharose chromatography of CHAPS-solubilized pig liver membranes. Pig liver membrane extracts, solubilized with 0.4% CHAPS, were applied to DEAE-Sepharose (5 × 20 cm). A NaCl gradient (···, linear 40–400 mM, 750 ml; isocratic 400 mM, 250 ml; linear 400–750 mM, 500 ml) was used for protein elution and fractions of 12 ml were collected. Aliquots were assayed for [^{125}I]VIP (▲) and [^{125}I]glucagon (△) total binding activities as described in section 2 and binding is expressed as fmol/ml. Absorbance at λ = 280 is shown by the broken line (---). Fractions used for further studies were named A, B, C and D in their eluting order as indicated by the bars.

A were eluted at 40 mM NaCl buffer, whereas the others required increasing concentrations of NaCl. A VIP and glucagon degrading activity was present in fraction B. Aliquots (100 μl) of fraction B were incubated with [^{125}I]VIP or [^{125}I]glucagon (20 pM) for 2 h at room temperature. After trichloroacetic acid (10%) precipitation and centrifugation (3,000 × g for 15 min), the radioactivity of the supernatant was determined, as a crude index of peptide degradation and corresponded to 50% and 58% of the initial radioactivity for glucagon and VIP, respectively. When [^{125}I]glucagon and [^{125}I]VIP were incubated with fraction A, the index of degradation was 3% and 21%, respectively. Similar values were obtained when the labeled peptides were incubated with 20 mM Tris-HCl (pH 7.5) buffer only. Both [^{125}I]VIP and [^{125}I]glucagon binding activities of fraction C could not be inhibited by excess native peptides (5 μM) indicating that the binding is non-specific.

3.3. *Binding of [^{125}I]VIP and [^{125}I]glucagon to proteins of DEAE fractions A and D*

3.3.1. [^{125}I]VIP binding sites of fraction A

Concentrations of VIP that induced half-maximal inhibition (IC_{50}) of [^{125}I]VIP binding to fraction A (Fig. 2A), were 1.8 ± 0.5 nM indicating the presence of high-affinity VIP binding proteins. A Scatchard plot [22] of the competition data and analysis using the 'Ligand program' [23] indicated the presence of a single high affinity site for VIP with a dissociation constant (K_d) of 1.5 ± 0.6 nM and a maximum binding site concentration (B_{max}) of 1.3 ± 0.4 pmol/mg. The potency order of

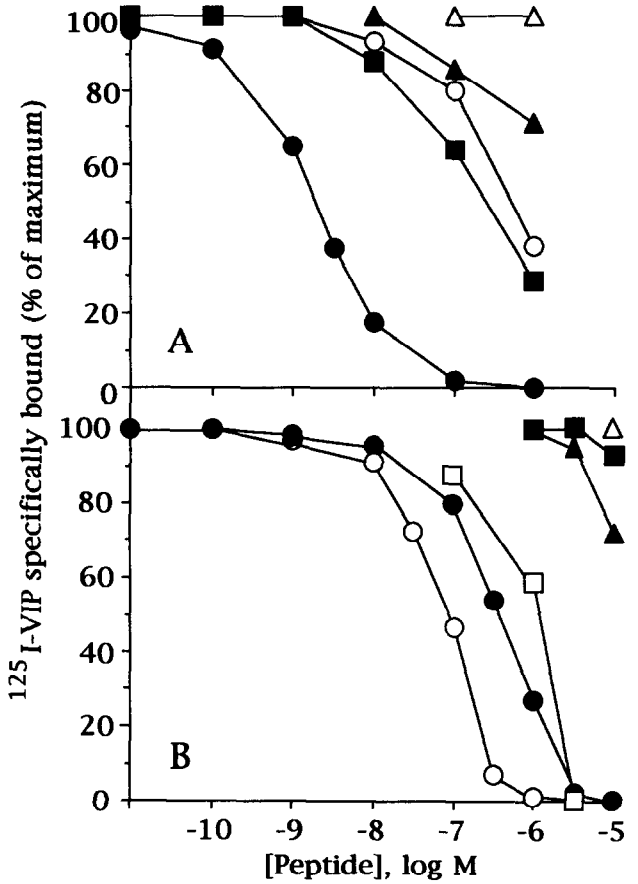

Fig. 2. Inhibition of [^{125}I]VIP binding to soluble proteins separated by DEAE chromatography. Proteins in DEAE fraction A (panel A) and fraction D (panel B) were incubated with [^{125}I]VIP (20 pM) and increasing concentrations of unlabeled VIP (●), rGRF (○), PHI (■), secretin (▲), VIP(10–28) (□) or glucagon (△). Results are expressed as percentage of specific binding, determined as described in section 2, in the absence of unlabeled peptides. Each point represents the mean of three experiments performed in duplicate.

peptide inhibition of VIP binding is: VIP > PHI > rGRF > secretin (Fig. 2A).

3.3.2. [^{125}I]VIP binding sites of fraction D

[^{125}I]VIP binding to fraction D (Fig. 2B) was inhibited by VIP in a dose-dependent manner, but the IC_{50} value of 0.7 μM was about 400-fold higher than that obtained with fraction A. A different peptide specificity was also obtained, the order of potency being rGRF > VIP > VIP(10–28) > secretin > PHI.

3.3.3. [^{125}I]glucagon binding sites of fraction A

[^{125}I]glucagon binding was dose-dependently inhibited by the extended form of glucagon, oxyntomodulin (pig or human), which is almost 100-fold less potent than glucagon (Fig. 3). Neither GLP-1(17–36) nor GLP-2 affected the binding of [^{125}I]glucagon to fraction A. The presence of a single high affinity site for glucagon (K_d and B_{max} values were 0.08 ± 0.03 nM and

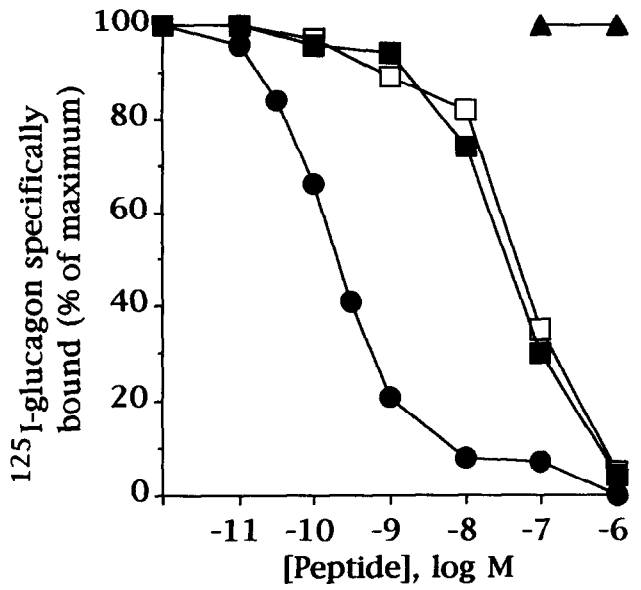

Fig. 3. Inhibition of [^{125}I]glucagon binding to soluble proteins separated by DEAE chromatography. Proteins in DEAE fraction A were incubated with [^{125}I]glucagon (20 pM) and increasing concentrations of unlabeled glucagon (●), human oxyntomodulin (■), pig oxyntomodulin (□), GLP-1(7–36) (▲) or GLP-2 (▲). Results are expressed as percentage of specific binding, determined as described in section 2, in the absence of unlabeled peptides. Each point represents the mean of three experiments performed in duplicate.

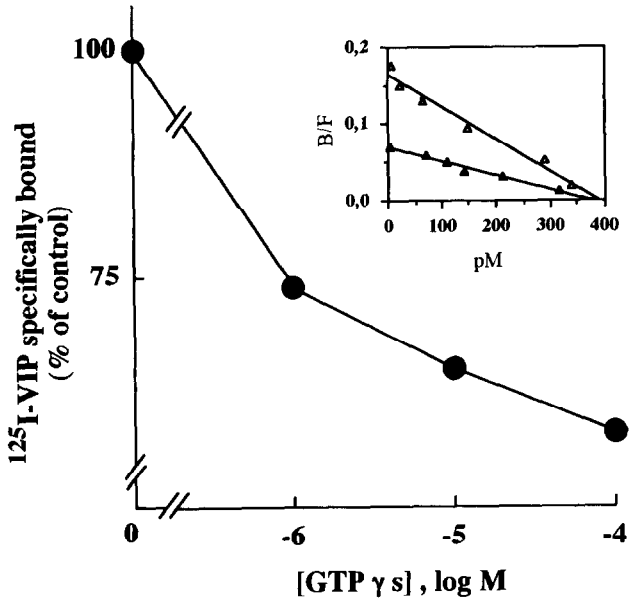

Fig. 4. Effect of GTP-γ-S on the [^{125}I]VIP binding to fraction A from DEAE chromatography. Proteins from DEAE fraction A were incubated with 20 pM of [^{125}I]VIP with increasing concentrations of GTP-γ-S. Data are expressed as percentage of specific binding, determined as described in section 2, in the absence of GTP-γ-S. Each point is representative of two experiments performed in duplicate. Inset: Scatchard plot of VIP binding in the absence (△) or presence (▲) of 20 μM GTP-γ-S.

0.31 ± 0.01 pmol/mg, respectively) was obtained by analysis of the competition data of fraction A.

3.3.4. Effect of GTP-γ-S on the [^{125}I]VIP binding

The non-hydrolyzable GTP analogue, GTP-γ-S, inhibited the [^{125}I]VIP binding in fraction A in a dose-dependent manner and 45% inhibition of binding occurred at 100 μM (Fig. 4). At this concentration GDP had a similar inhibitory effect (40%) while ATP did not effectuate binding. Further, competitive binding studies in the presence of 20 μM GTP-γ-S induced a shift in the affinity from 1.5 to 6.5 ± 1.1 nM (Fig. 4). GTP-γ-S, up to 100 μM, had no inhibitory effect on the [^{125}I]VIP binding to fraction D or [^{125}I]glucagon binding to fraction A.

3.4. *SDS-PAGE of [^{125}I]VIP cross-linked to proteins of DEAE fraction D*

Proteins separated by DEAE-chromatography in fraction D were cross-linked to [^{125}I]VIP using disuccin-

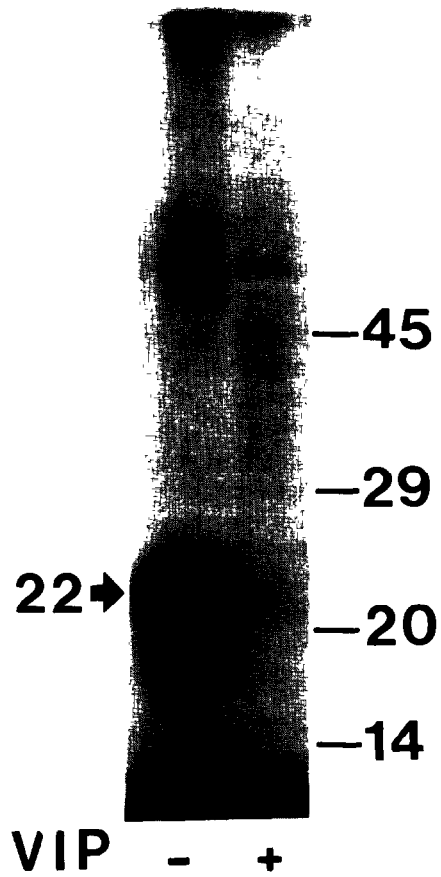

Fig. 5. SDS-PAGE autoradiographs of the low-affinity [^{125}I]VIP binding proteins separated by DEAE chromatography. [^{125}I]VIP was cross-linked with disuccinimidyl suberate to soluble proteins (fraction D) in the presence (+) or the absence (−) of 5 μM VIP. Samples were resolved on SDS-PAGE (10–15% gel). The resulting autoradiographs are shown with the migration position of protein markers (kDa) indicated on the right and the arrow indicating the molecular masses (kDa) of the major labeled proteins.

imidyl suberate and analyzed by SDS-PAGE and autoradiography (Fig. 5). A labeled band corresponding to a size of 22 kDa was identified and the labeling specifically inhibited by 5 μM VIP.

3.5. *Purification and characterization of low VIP affinity binding sites of the DEAE fraction D*

Fraction D, which unlike the high affinity proteins showed no adsorption to wheat-germ-agglutinin agarose, was further purified by gel filtration, affinity chromatography, and a final anion-exchange chromatographic step on Mono Q. Fractions containing specific [^{125}I]VIP binding were pooled for further processing. A single band of protein, with a molecular mass of 18 kDa, estimated by SDS-PAGE analysis and silver staining, was obtained after the final purification step (Fig. 6). This purification recovered 0.5 mg of this protein from the starting 2.4 g of solubilized membrane protein. Initial attempts to sequence the protein were unsuccessful probably due to a blockage of the N-terminal residue. Two nmol protein was therefore enzymatically hydrolyzed by endoproteinase Lys-C and the resulting fragments were separated by reversed phase HPLC. The major peak had the sequence of D-G-N-G-Y-I-S-A-A-E-L-R-H-V-M-T-N-L-G-E-X-L-T-D-E-E-V-D-E-M-I-R-E-A- (X representing an unidentified amino acid) which is identical to mammalian calmodulin(95–128). The low-affinity VIP binding protein furthermore exhibited a [^{125}I]VIP binding similar to that of pig brain calmodulin. Competitive inhibition of [^{125}I]VIP binding by native VIP resulted in similar IC_{50} values, 0.3 μM and 0.2 μM, respectively. Also the calmodulin inhibitor, calmidazolium inhibited the [^{125}I]VIP binding to the purified protein and calmodulin similarly with an IC_{50} concentration of 2 μM.

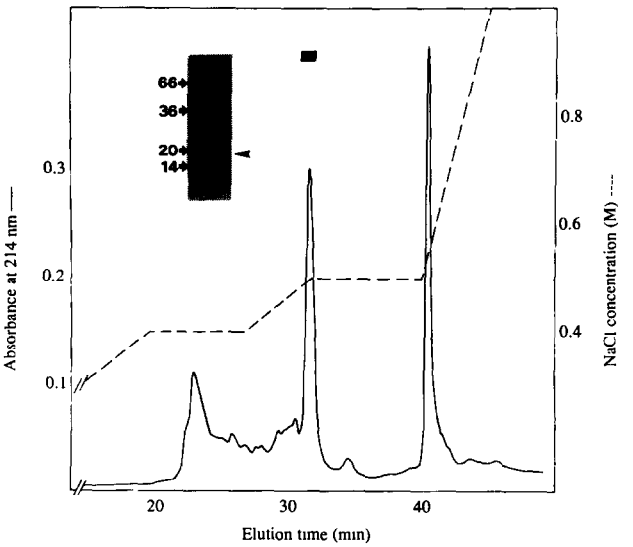

Fig. 6. Purification of low affinity VIP-binding protein (fraction D) by anion-exchange chromatography. VIP-binding proteins in fraction D were purified by gel filtration and VIP-affinity chromatography and concentrated as described in section 2. The sample was injected on a Mono Q 5/5 column equilibrated in 20 mM Tris-HCl, pH 7.0, 0.1% CHAPS at a flow rate of 1 ml/min. Bound proteins were eluted with a gradient (0–1 M) NaCl (---) and the elution of the proteins was monitored at λ 214 nm (—). Inset: the fractions containing [^{125}I]VIP binding activity, indicated by the bar, were pooled and subjected to SDS-PAGE analysis. The migration positions of protein markers (kDa) are shown on the left of the silver-stained gel and the migration of the single band stained is shown on the right.

4. DISCUSSION

This simultaneous solubilization and initial purification of VIP and glucagon high affinity binding proteins is reported. DEAE-chromatography separated high affinity binding proteins for VIP and glucagon (fraction A) from proteins which bound glucagon (fraction B) and VIP (fraction D) with a lower affinity.

The putative VIP and glucagon receptors co-eluted in the same fraction (A) with binding activities of 1.3 pmol/mg for VIP and 0.31 pmol/mg for glucagon. Thus, a 2-fold purification was estimated after the DEAE chromatography step with a 35–40% yield of VIP and glucagon receptors, respectively. The VIP binding sites show an affinity for VIP (K_d 1.5 nM) comparable to that of the plasma membrane receptor (K_d 1.7 nM) and have a similar peptide specificity: VIP > PHI > rGRF > secretin. The affinity of [^{125}I]VIP binding protein in fraction A is reduced by 4-fold with GTP-γ-S suggesting that it is functionally associated with a G protein. In the presence of GTP-γ-S the binding affinity (K_d 6.5 nM) of VIP is similar to that of GTP-insensitive VIP-receptors solubilized from pig liver by Voisin [12]. As in our study, high-affinity VIP receptors in solubilized rat liver cell membranes maintain their GTP sensitivity [9].

The solubilized [^{125}I]glucagon binding proteins displayed the same peptide specificity and K_d values (0.08 nM) as those observed for glucagon receptors in pig liver cell membranes (0.12 nM) [24]. Both VIP and glucagon receptor protein complexes from this DEAE fraction (A) were found to contain *N*-acetylglucosamine and/or sialic acid residues in agreement with other reports on hepatic VIP [25] and glucagon [10] receptors. These findings indicate the presence of distinct high affinity VIP and glucagon binding proteins in the DEAE-purified fraction A.

The biological significance of VIP binding directly to calmodulin is unclear although VIP and VIP(10–28) have been shown to be potent inhibitors (IC_{50}: 0.5 μM) of calmodulin stimulation of brain phosphodiesterase activity [26]. Further, there is a close association between the regulatory roles of calmodulin- and VIP-induced signal transduction. Indeed, trifluoroperazine and an anti-calmodulin serum have been shown to reduce VIP-enhanced adenylate cyclase activity by 70% in pituitary adenoma GH_4C_1 cells [27] and calmodulin-sensitive adenylate cyclase has been demonstrated in several tissues [28].

Further studies are necessary to ascertain if a calmodulin-like protein can be associated with the VIP receptors as postulated for insulin receptors, which have been suggested to contain a calmodulin-binding domain [29].

In conclusion, the simultaneous solubilization of active high affinity VIP and glucagon putative receptors has been achieved as well as their separation from other VIP and glucagon binding proteins by anion-exchange chromatography. Furthermore, the binding of VIP to calmodulin in liver cell membranes may suggest that calmodulin could be involved in the regulation of VIP activity.

While this manuscript was in preparation, the cloning of two nonidentical high-affinity receptors for VIP was reported [30,31]. In one of the cases [31], expression of receptor mRNA was observed in the liver. Also a report appeared according to which a membrane-bound VIP-binding protein from guinea pig lung had been identified as calmodulin (D. Stallwood et al. (1992) J. Biol. Chem. 267, 19617–19621).

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REFERENCES

[1] Said, S.I., Vasoactive intestinal peptide, in: Advances in Peptide Hormone Research series, Vol. 1, Raven Press, New York, 1982, pp. 1–512.
[2] Feliu, J.E., Mojena, M., Silvestre, R.A., Monge, L. and Marco, J. (1983) Endocrinology 112, 2120–2127.
[3] Hagopian, W.A. and Tager, H.S. (1984) J. Biol. Chem. 259, 8986–8993.
[4] Lotersztajn, S., Pavoine, C., Brechler, V., Roche, B., Dufour, M., Le-Nguyen, D., Bataille, D. and Pecker, F. (1990) J. Biol. Chem. 265, 9876–9880.
[5] Rosselin, G., in: Handbook of Physiology, The Gastrointestinal System, Neural and Endocrine Biology (G. Schultz, G.M. Makhoulf and B.B. Rauner, Eds.), II, American Physiological Society, Bethesda, MD, 1989, pp. 245–280.
[6] Desbuquois, B. (1974) Eur. J. Biochem. 46, 439–450.
[7] Padrell, E., Herberg, J.T., Monsatirsky, B., Floyd, G., Premont, R.T. and Iyengar, R. (1987) Endocrinology 120, 2316–2325
[8] Couvineau, A., Voisin, T., Guijarro, L. and Laburthe, M. (1990) J. Biol. Chem. 265, 13386–13390.
[9] Guijarro, L., Couvineau, A., Calvo, J.R. and Laburthe, M. (1989) Regul. Pept. 25, 37–50.
[10] Herberg, J.T., Codina, J., Rich, K.A., Rojas, F.J. and Iyengar, R. (1984) J. Biol. Chem. 259, 9285–9294
[11] McVittie, L.D. and Gurd, R.S. (1989) Arch. Biochem. Biophys. 273, 254–263.
[12] Voisin, T., Couvineau, A., Guijarro, L. and Laburthe, M. (1991) Life Sci. 48, 135–141.
[13] Paul, S. and Said, S.I. (1987) J. Biol. Chem. 262, 158–162.
[14] Brugger, C.H., Stallwood, D. and Paul, S. (1991) J. Biol. Chem. 266, 18358–18362.
[15] Said, S.I. and Mutt, V. (1970) Science 169, 1217–1218.
[16] Tatemoto, K. and Mutt, V. (1981) Proc. Natl. Acad. Sci. USA 78, 6603–6607.
[17] Jorpes, J.E., Mutt, V., Magnusson, S. and Steele, B.B. (1962) Biochem. Biophys. Res. Commun. 9, 275–279.
[18] Couvineau, A. and Laburthe, M. (1985) Biochem. J. 225, 473–479.
[19] Laemmli, U.K. (1970) Nature 227, 680–685.
[20] Andersson, M., Marie, J.-C., Carlquist, M. and Mutt, V. (1991) FEBS Lett. 282, 35–40.
[21] Person, W.R. and Lipman, D.J. (1988) Proc. Natl. Acad. Sci. USA 85, 2444–2448.
[22] Scatchard, G. (1949) Ann. NY Acad. Sci. 51, 660–672.
[23] Munson, P.J. and Rodbard, D. (1980) Anal. Biochem 107, 220–239.
[24] Baldissera, F.G.A., Holst, J.J., Knuhtsen, S., Hilsted, L. and Nielsen, O.V. (1988) Regul. Pept. 21, 151–166.
[25] Couvineau, A., Rouyer-Fessard, C., Voisin, T. and Laburthe, M. (1990) Eur. J. Biochem. 187, 605–609.
[26] Barnette, M.S. and Weiss, B. (1985) J. Neurochem. 45, 640–643.
[27] Gordeladze, J.O., Sletholt, K., Thorn, N.A. and Gautvik, K.M. (1988) Eur. J. Biochem. 177, 665–672.
[28] Rosenberg, G.B. and Storm, D.R. (1987) J. Biol. Chem. 262, 7623–7628.
[29] Graves, C.B., Goewert, R.R. and McDonald, J.M. (1985) Science 230, 827–829.
[30] Sreedharan, S.P., Robichon, A., Peterson, K.E. and Goetzl, E.J (1991) Proc. Natl. Acad. Sci. USA 88, 4986–4990.
[31] Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. and Nagata, S. (1992) Neuron 8, 811–819.