glycine were measured by the method of Lowry et al., of Rojkind and Gonzalez and of Ohmori et al., respectively[5-7]. All 3 constituents were determined in samples of livers excised before hepatic perfusion.

Results and discussion. The hepatic content of proline increased significantly over control and sham values following duct ligation (table 1). The glycine content, in contrast, was not affected by the ligation procedure (table 2). Uptake of both labeled proline and glycine by hepatocytes and by duct cell enriched fractions was significantly higher than in controls. The proline incorporation in the duct cell fraction was not higher than that in hepatocytes except on day 3 after ligation (table 1). Although a previous study showed that ^{3}H proline appeared to be more concentrated in bile ducts and fibroblast-like cells than in parenchymal cells[8], we did not find a consistent comparative increase in uptake by ductular cells. This may have been due to the heterogeneity of the isolated cell fraction. The radioactive counts of proline and glycine in the 2 cell fractions are extremely low, representing on the average less than a millionth part of the injected doses. This may be attributed in part to the loss of the water soluble amino acids during the isolation procedures and in part to the active secretion of the labels out of the liver. The accelerated metabolism of both proline and glycine in experimental compared to control rats is to be expected with the striking proliferation of hepatocytes and ductular cells that occurs 1 to 2 days after extrahepatic cholestasis[9]. Our results suggest that the increased hepatic content of proline following obstruction of the bile duct is a mechanism for the duct hyperplasia. The mediator role of the amino acid is analogous to that shown for the hyperplastic response in experimental fascioliasis. As proline also regulates collagen production in both human and experimental cirrhosis[10,11], it accounts for the concurrent development of fibrosis and duct hyperplasia that occur in cirrhosis.

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Extraction of two different protein kinase activities from bovine rod outer segments

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Summary. An optimization of the rod outer segment (ROS) preparation technique is described. The protein responsible for ATP-γ ^{32}P binding to bovine ROS was separated from the protein active with protamine on a DEAE Sephadex column. Molecular weight evaluation on a G 100 Sephadex column gave a value of 75,000 for the protein active with ROS, and 42,000 for that active with protamine. 1.25 mM c-AMP or c-GMP reduced the activity to 0.7 or 0.8 of the control respectively. 10 mM c-GMP doubled the yield of the active protein extracted from ROS.

More than 10 years ago, it was observed that incubation of prepared rod outer segment (ROS) membranes of the vertebrate retina with adenosine 5'-[γ-^{32}P]triphosphate (γ-^{32}P-ATP) in light resulted in labeling of the ROS with ^{32}P. Many authors who have studied this phenomenon have assumed that ATP phosphorylates rhodopsin on the ROS membrane after light activation of the substrate and that a protein, possibly weakly bound to ROS, is active in this reaction: the 'rhodopsin kinase'[1-7]. Attempts to isolate and characterize this active protein have also been made. Kühn postulated a mol. wt of 67,000-69,000 while Shichi and Somers[8] estimated it to be 50,000-53,000. To clarify this discrepancy, we publish some of our experimental observations on this active protein, made 3 years ago. In this study we discuss the preparation of bovine ROS with a maximal content of active protein, optimal extraction of the active protein from prepared ROS, its separation on a DEAE Sephadex column, molecular weight determination, and the effect of several ions and cyclic nucleotides on the protein activity and extraction.

Materials and methods. The technique for preparing bovine outer segments from freshly dissected retinae was a modification of a preceding technique[9] with the aim of increasing the yield (cf. results). Flotation and sucrose density gradient centrifugation steps in the dark remained unaltered, but the p[H^+] value was established at 5.9 and the concentration of phosphate buffer was increased to 0.143 M. Dithiothreitol (DTT, 20711, Serva, Heidelberg, FRG) was omitted because it could inhibit the active protein; 0.1 mM phenylmethylsulfonylfluoride (PMSF, Serva, 32395) and 0.31 M K Cl were present in every solution.

Extraction of water-soluble proteins from ROS. ROS from 30 to 35 eyes were gently homogenized with 1.0 ml 0.1 mM PMSF, added with 0.125 ml 140 mM phosphate elution buffer (see below), and centrifuged at $15.10^3 \times$ g. This procedure was repeated 3 times on the same pellet. The 4 supernatants were collected concentrated and equilibrated on an Amicon PM 10 membrane in a microultrafiltration cell; the final volume was less than 1 ml.

Capacity of soluble ROS protein to bind ATP-γ^{32}P to inactivated ROS or protamine was tested in a mixture of the following composition. In a polypropylene 1.5 ml micro test tube, 0.050 ml 3.6 mM γ-^{32}P-ATP (6000 dpm/nmole) (Amersham Buchler Braunschweig, FRG), 0.200 ml 132 mM KH_2PO_4 adjusted with KOH to p[H^+] = 7.89 and containing 8 mM $MgCl_2$; 0.100 ml of the solution to be tested and 0.050 ml alum-ROS suspension (see below) containing 50-60 nmoles rhodopsin in 1.00 ml or 0.050 ml

protamine (2 mg · ml, Sigma, protamine sulfate P 4020) solution in test buffer were added in the dark; the test tubes were then illuminated with a 50 W tungsten bulb at a distance of 15 cm for 15 min at 37 °C. ATP-γ^{32}P binding to protamine was not influenced by illumination.

The reaction was stopped with 100 µl 30% (w/v) Cl_3CCOOH solution at 0 °C. After centrifugation at 500 × g, the sediment was washed (according to U. Wilden, 1978, personal communication) by shaking with the same Cl_3CCOOH solution after a further centrifugation; the washing procedure was repeated twice. The incorporation of ATP-γ^{32}P into alum-ROS amounted to about 0.2 moles pro moles rhodopsin; the incorporation in a parallel assay incubated in the dark was thereby subtracted. Alum-ROS were ROS homogenized in glass-Teflon homogenizer with 4% KAl $(SO_4)_2 \cdot 12H_2O$ (alum) and successively washed with test phosphate buffer using a similar homogenizer.

Fluorometric determination of protein concentration was carried out by adding 0.500 ml of a solution of 30 mg fluorescamin (Serva 21551) in 100 ml dioxan (Merck 2967) to 1.60 ml of the sample containing 5-20 µg protein in 50 mM phosphate elution buffer. Excitation radiation was at 365 nm and emission light at 475 nm.

Chromatographic separation on ion exchanger column. Bio-Rad Econo-Columns (diameter 0.7 cm, length 6 cm) were used. Ion exchanger: DEAE Sephadex A 25 equilibrated with phosphate buffer (15 mM KH_2PO_4 adjusted with KOH to $p[H^+]=7.89$). After application of the sample equilibrated on an Amicon PM 10 membrane with the buffer above, containing 0.1 mM PMSF, elution was performed at first with 7.0 ml of the same equilibration buffer, and then with 35.0 ml of a linear gradient from 15 mM up to 140 mM phosphate buffer of similar composition followed by 10.0 ml 140 mM phosphate buffer and 20 ml 1 M KCl.

Gel filtration for determining apparent molecular weight was performed on Sephadex G 100 superfine; column size: diameter 1.0 cm, length 42 cm. Elution buffer was 70 mM Na-phosphate at $p[H^+]=7.0$ containing 1 mM $MgCl_2$. Bovine serum albumin (Serva 11920, mol. wt = 67,000) and horse myoglobin (Serva 29895, mol.wt = 17,800) were used as calibration proteins.

Results. Optimization of the ROS preparation technique. The process was carried out at different $p[H^+]$values. Remarkably, most activity (about 93%) was lost in the initial procedure[9] (cf. table 1). ROS suspensions (the first one, A, as supernatant after retina sedimentation, the second one, B, as suspension of the ROS previously separated via the sucrose density gradient) were also tested; the active protein in the activity assay could also bind ATP-γ-^{32}P to the intact ROS of the suspension when no alum-ROS were added. This activity was designated internal activity (phosphorylation of endogenous proteins, to which Fesenko et al.[10] referred, need not be considered in this case because binding of ATP-γ-^{32}P in the dark was subtracted). By adding alum-ROS to the assay in these cases, a higher ^{32}P incorporation in total ROS was observed. With regard to the internal activity (about 11% of the total activity in A) one third of the initial activity was found in the prepared ROS. These findings revealed that a) the most active protein (about 89%) was already unable to reach the binding sites for ATP-γ-^{32}P in intact ROS at the first step of the preparation (suspension A) in the assay, b) most (93%) of the active protein was eluted during the procedure of ROS preparation, and half of the activity of the prepared ROS was internal activity.

In attemping to improve this unfavorable situation, it was observed that the protein concentration in the supernatant of suspension A depended on $p[H^+]$ value; 3.8, 7.5, or 8.2 mg · ml at $p[H^+]=6.6$, 7.0, or 7.5 respectively. PMSF protected the active protein. The procedure described here is advantageous: a) a greater part (23%) of the total active protein in suspension A could be tested as internal activity in the assay; b) the yield amounted to 35% for the total active protein, and to 28% for the internal activity. To summarize, the modification of the initial procedure essentially allowed a greater internal activity to be conserved already at the first step (A) of the preparation procedure.

Experiments on extraction of the active protein from ROS were performed with the aim of obtaining the active protein quantitatively. By extraction steps on the same ROS preparation (from about 30 eyes), consisting of homogenization with 5.0 ml H_2O, successive centrifugation at 15×10^3g and addition to the supernatant of 0.6 ml 140 mM phosphate extraction buffer, the protein solubilized in the first step amounted to 3.5 mg, in the second 2.1 mg and in the third step 0.35 mg.

Taking the amount of the active protein extracted at the first step as equal to one, the amount at the second step was 0.8, and at the third 0.3. In parallel experiments in which the extraction medium contained 2 mM DTT, the extracted

Table 1. Protein kinase activities relative to protein kinase activity in a retinal suspension A

	Flotation of the retinae in suspension A			ROS preparation	
	External	Internal	Total	Internal	Total
Initial procedure	0.89	0.11	1.0	0.035	0.07
Optimized procedure	0.77	0.23	1.0	0.065	0.35

Active protein yield of ROS preparation at different steps of the initial and the optimized procedure.

Table 2. Extractions of equal quantities of prepared ROS with different solutions (cf. 'Results and discussion')

Extraction solution	Relative activity in the extract	
	In light	In the dark
H_2O	0.92	0.98
70 mM phosphate buffer[9]	1.0	1.0

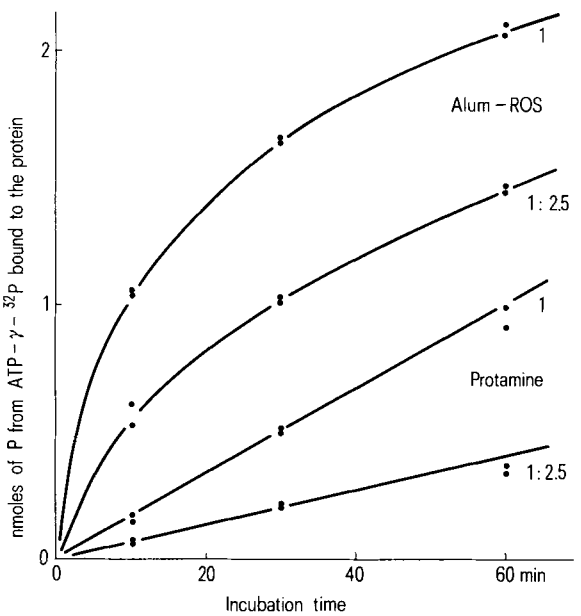

Figure 1. Progress curves of ATP-γ-^{32}P incorporation in alum-ROS or protamine by adding ROS extract (1) or a dilution (1:2.5) of it.

activity was always smaller than that in the extraction without DTT; 0.9 in the first step, 0.6 in the second step and 0.1 at the third. After 7 extraction steps, there was no active protein on extracted ROS. The sum of extracted active protein gave 30% less activity for extraction with DTT. The total extracted protein quantity was unaffected by DTT.

In an attempt to compare the extraction procedures performed here, a ROS sediment corresponding to the preparation of about 25 eyes was gently homogenized with 0.42 M sucrose containing 35 mM phosphate buffer for the preparation procedure. Eight aliquot portions were prepared, and centrifuged at 15×10^5 g. The sediments were then extracted in the dark and in the light respectively by using 1.00 ml. H_2O or 70 mM phosphate buffer for the initial preparation procedure[9]. The yields of activity, tested according to 'Materials and methods', are shown in table 2.

Standardization of test conditions in the assay for determining the activity to bind ATP-γ-^{32}P to alum-ROS. For this purpose, buffer substance and p[H^+]value, and alum-ROS quantity were varied. The assay conditions chosen here were confirmed substantially by Chader et al.[11,12] who also focussed their attention on the effect of phosphate ions. At p[H^+] = 7.9 the activity in the assay performed according to 'Materials and methods' was about 20% greater than in the same assay in which phosphate buffer was replaced with tri(hydroxymethyl) aminomethane (0.10 M)-phosphate buffer or triethanolamine (0.10 m)-HCl buffer at the same p[H^+]value. The activity depending on phosphate ion concentration increased with the phosphate content and was maximal at 0.07 M. The dependence of the activity on the alum-ROS quantity in the assay was an increasing linear function at least up to the rhodopsin content of 23 nmoles pro ml in the test. The measured activity is not a linear function of the ROS extract quantity in the assay; it is possibly a quadratic function.

Progress curves for ATP-γ-^{32}P incorporation in alum ROS or protamine vs time are shown in figure 1. The assays with alum-ROS or protamine were carried out under standard conditions by adding ROS extract or a dilution (1:2.5) of it. It is striking that linear time dependence was not observed for the incorporation of ^{32}P in the protein during an incubation time of 60 min. In the case of protamine, linearity could be assumed for the first 15 min, whereby the ^{32}P quantity incorporated was proportional to the ROS extract content. The 2 activities could be separated (cf. Chromotographic Separations below).

The effect of several ions on the active protein in the assay was tested in the ROS extract according to Shichi[8] and the standard procedure in this paper. The results are shown in table 3. Assays containing the active protein from a DEAE Sephadex or Sephadex G 100 column yielded no significant results.

Besides the experiments with (alum) denatured ROS, it was observed that the activity in intact discs prepared according to Smith et al.[13] was not influenced by KCl or NaCl (both 29 mM).

The effect of cyclic nucleotides on ATP-γ-^{32}P binding to alum-treated ROS, membranes on assay conditions and on extractibility of protein kinase activities in illuminated ROS was investigated. Our results contrast with those of Chader et al.[12], but are in agreement with Fesenko and Orlov[14] (both groups used Tris-HCl buffer). If the activity assay contained 1.25 mM cAMP or 1.25 mM cGMP, the activity measured amounted to 0.7 and 0.8 of the control respectively. Light extraction (15 min in front of a 60 W tungsten bulb at 20–25 °C) under standard conditions was not influenced by cAMP (10 mM in extraction solution), but the yield was doubled if cGMP was present at a concentration of 10 mM.

Stability of the active protein in ROS extract was observed in solutions consisting of diluted (1:2) ROS extract prepared according to 'Materials and methods', and containing ATP (0.6 mM), phosphate ions (140 mM) or MgCl (0.6 mM) respectively. Phosphate ions allowed recovery of 98% (ATP and/or $MgCl_2$ 80%) of the activity after 1 day, 90% (ATP and/or $MgCl_2$ 60%) after 3 days, and 75% (ATP and/or $MgCl_2$ 40%) after 5 days; the simultaneous presence of all these compounds was not more efficient. A loss of active protein could already be calculated from determinations carried out during the preparation of ROS; this loss amounted to about 27% (related to the activity in A) for the preparation procedure of Kühn[9], and about 42% for the procedure described here (it must be considered that the internal activity was not and could not be measured under standard conditions). PMSF, which protected the active protein during preparation steps, was less effective in the extract from prepared ROS.

Chromatographic separation of the active protein on a DEAE Sephadex A 25 column. Figure 2 shows an example of such a separation. Fractions were thereby achieved which had a 5-fold greater specific activity (in relation to protein weight) than the ROS extract, but their activity was very labile. Neither bovine serum albumin (1 mg · ml), nor about 70 mM phosphate elution buffer could stabilize it. A successive chromatographic separation could not be carried out, because no activity could be established after any

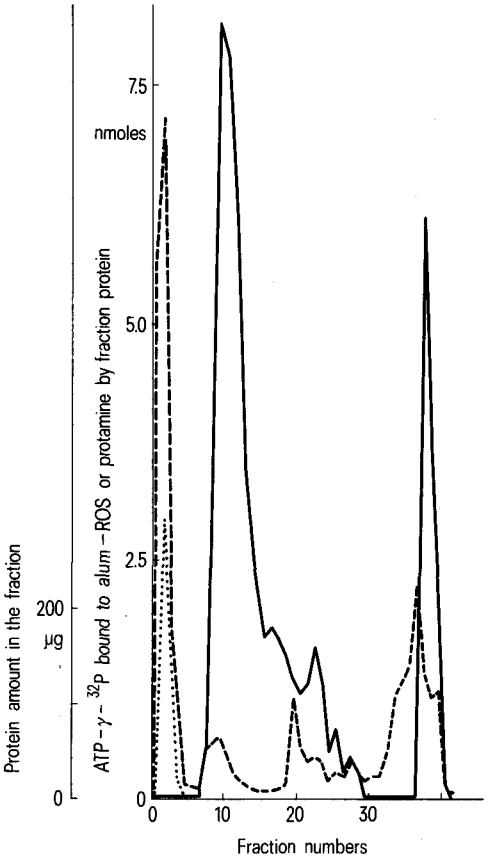

Figure 2. Elution profile on a DEAE Sephadex column showing protein kinase activity of a ROS extract. In the example in the figure, 3.37 mg extract protein were applied. On the ordinate, the capacity of the whole fraction protein to catalyze binding of ATP-γ-^{32}P on alum-ROS (——) or protamine (.....) is specified. For further explanations cf. the text and 'Materials and methods'. The gradient was started by collecting fraction 5; fractions 26 to 32 were eluted with 140 mM phosphate buffer, successively with 1 M KCl.

further separation procedure. Quick freezing in liquid nitrogen did not permit conservation either. The capacity to bind ATP-γ-^{32}P to protamine was not retained in the fractions which showed the capacity to bind ATP-γ-^{32}P to alum ROS. The protein active with protamine was not retained by the column and was stabile. The capacity to bind ATP-γ-^{32}P to alum ROS lost through the chromatographic separation could be restored again partially (50%) in the active fractions by adding ROS extract. Parallel to the activity test for the fractions, a similar test was carried out by adding 0.050 ml of diluted ($^1/_4$) ROS extract, which was applicated on the column for the separation.

No presence of phosphatase activity, which was demonstrated by Miller and Paulsen[15] in frog, could be observed. For this assay, alum-ROS were bound with ATP-γ-^{32}P under the conditions of the activity assay and then washed with the assay buffer and again suspended in it. Equal portions containing 2.5 nmoles rhodopsin were added to 0.100 ml of every fraction and incubated in the dark 15 h at 25 °C. No fraction was able to release the ^{32}P bound on alum-ROS previously incubated with γ-^{32}P-ATP and ROS extract.

Chromatography of a ROS extract on a Sephadex G 100 superfine column calibrated with dextran blue (Serva, 18706), horse myoglobin and bovine serum albumin showed a mol.wt of 75,000 for the protein active with alum-ROS and of 42,000 for that active with protamine. A minor part (20%) of the activity with protamine was found in the fractions in which dextran blue was to be found. Some modifications in the extraction procedure of the soluble proteins from ROS were not able to induce modifications of these apparent molecular weights. Apart from the extraction procedure in 'Materials and methods', the following procedure was also performed: ROS containing 25 mg rhodopsin was homogenized with 0.75 ml 1 mM $MgCl_2$, 0.1 mM PMSF, 2 mM DTT at 0 °C; after 10 min at this temperature, 0.16 ml 0.40 M Na phosphate ($p[H^+]=7.0$) were added and a supernatant was produced at 15,000 × g. In similar experiments, this extract was successively equilibrated with 0.30 M K phosphate at $p[H^+]=7.5$ or with 15 mM K phosphate at $p[H^+]=7.89$ on a PM 10 membrane.

Discussion. The active protein which promotes the light-induced ^{32}P-labeling of ROS rhodopsin on incubation with γ-^{32}P-ATP can exist in a water-soluble or membrane-associated form[9]. In the dark, it is extractable in water; consequently, a major part of the activity was lost during the ROS preparation. By using a lower $p[H^+]$-value (=5.9) and higher ionic strength (0.31 M KCl) we obtained a better yield of active protein in prepared purified ROS. Shichi and Somers[13] worked with a crude ROS preparation which corresponds approximately to that at the step before sucrose density gradient centrifugation in our technique. More highly purified ROS preparations were obtained by Fesenko et al.[16] and Livrea et al.[17]; these authors did not attempt to minimize the loss of internal proteins. The possibility we have shown of enhancing the yield of active protein (especially as internal activity) in prepared ROS gives an indication of possible location in the ROS. Also the behavior of this active protein with cGMP during the extraction must be considered in this context.

Progress curves of ^{32}P label incorporation in alum treated ROS or protamine from γ-^{32}P-ATP (fig. 2) vs time, and the dependence of ^{32}P incorporation rate on the amount of ROS extract in the assay, show properties which give rise to the question whether an enzyme is directly concerned or not. Moreover, phosphate ions which inhibit kinases promote the activity of this protein. The partial reactivation of the diminished activity observed in the chromatographic fractions from the DEAE Sephadex column by means of whole ROS protein extract indicates the existence of a factor which participates in the activity. ^{32}P incorporation on protamine shows kinetics (cf. fig. 1) which are compatible with enzymic catalysis; the protein responsible could be separated from that active with alum ROS, and its mol. wt (42,000) was also determined. We could not reproduce all the results of Shichi and Somers[13] with regard to effects of several ions on the active protein (table 3). It is possible that our alum ROS and their urea-treated ROS are not the same, since the preparation techniques are different. A major discrepancy with the results of these authors concerns the mol. wt of the protein active with ROS which we found to be 75,000, and consistent with the value of 69,000 calculated by Kühn[9]. We have reproduced the gel filtration on a Sephacryl S-200 column[13] of the active protein with aldolase, bovine albumin, chymotrypsinogen A, and ribonuclease A as calibration proteins and determined a mol. wt of 79,000 for the protein active with ROS which is consistent with our finding (75,000). Therefore, we are inclined to explain the discrepancy as a mistake in the results of these authors. They also presented no evidence for the existence of activity in their polyacrylamide gel electrophoresis band at the molecular marker with mol. wt of 53,000. It is hence impossible to exclude the possibility that 50,000 is the mol. wt of a major impurity in their preparation. In our polyacrylamide gel electrophoresis (results not shown) of DEAE Sephadex or Sephadex G 100 column fractions containing protein active with ROS, no protein fraction with a mol. wt of 70,000–80,000 could be observed.

Table 3. Effect of different ions on the active protein

	Relative activity* in assay according to: Shichi (1978)	The standard procedure in this paper
Control	1.00	1.00
5′-AMP (1.0 mM)	0.54	0.13
Adenosine (1.0 mM)	0.53	0.75
$ZnCl_2$ (1.00 mM)	0.85	0.0
KCl (100 mM)	0.90	0.94
NaCl (100 mM)	1.03	0.88
Digitonin (1%)	0.0	0.0
Emulphogene (0.4%)	0.0	0.0

* Variation coefficients amounted to 0.07.

* The experiments were performed at the Institut für Neurobiologie of the Kernforschungsanlage Jülich GmbH, FRG. The author was supported by a grant from SFB 160 'Biologische Membranen' of Deutsche Forschungsgemeinschaft. The expert technical assistance of Miss Regina Augustin is gratefully acknowledged.

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Isolation and amino acid compositions of geographutoxin I and II from the marine snail *Conus geographus* Linné[1]

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Summary. The peptide toxins having inhibitory effects on the contractile response of the mouse diaphragm to direct stimulation, geographutoxin I and II, have been isolated from *Conus geographus*, and their amino acid compositions have been determined.

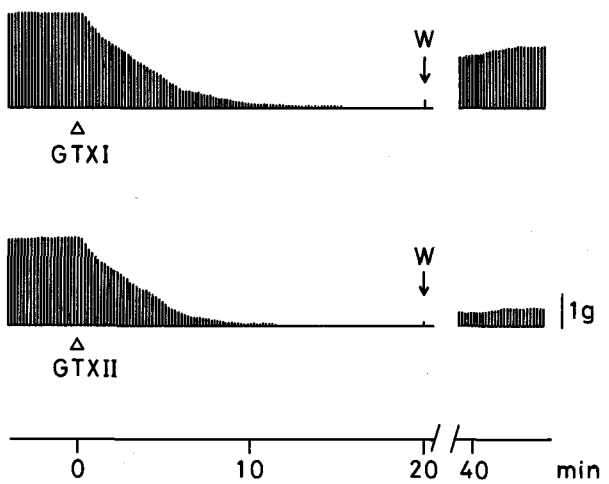
The species of the family Conidae use their specialized venom apparatus in the capture of prey organisms[2]. Among them, the venom of *Conus geographus* is most toxic to vertebrates and has been responsible for human fatilities[3]. It has been shown that the venom caused an inhibitory effect on the contractile response of directly stimulated isolated skeletal muscle[4-7]. Furthermore, preliminary purification of the substances having such a direct action on skeletal muscles has been reported[6]. Recently, 3 peptides having inhibitory actions on the post synaptic membrane of a vertebrate neuromuscular junction have been isolated from *C. geographus* and their amino acid sequences have been determined[8-10]. The present paper deals with the isolation and the amino acid compositions of 2 peptide toxins, geographutoxin (GTX) I and II from the venom of *C. geographus*.

Specimens of *C. geographus* were obtained from reefs in Okinawa waters. The gastropods were immediately frozen, shipped via air to Tokyo and stored at −20 °C until the specimens were used. The venom ducts (8.2 g) dissected from 30 animals (shell length 6.5–10.5 cm) were cut into small segments and the contents were squeezed out. The contents were extracted 3 times with 0.2 M acetic acid (80 ml each) and lyophilized to give a yellowish crude venom (1.2 g). The mouse isolated diaphragm preparation was set up and the contractile response to direct stimulation (0.1 Hz, 5 msec, supramaximal voltage) was measured as previously described[11]. Purification of the crude venom was carried out as the following procedure by monitoring the inhibitory effect on the diaphragm. The crude venom was chromatographed on a column of Sephadex G-50 (55 cm × 2.2 cm ID, Vo = 80 ml) using 1/30 M sodium phosphate buffer, pH 5.6, containing 0.15 M NaCl as eluant. The active portion (relative elution volume, Ve/Vo 1.7-2.9) was applied on a column of CM-Sephadex C-25 (50 cm × 2.6 cm ID) previously equilibrated with 1/30 M sodium phosphate buffer (pH 5.6) containing 0.15 M NaCl. The column was eluted with the same buffer (300 ml) and then with a linear gradient consisting of 1000 ml of 0.15 M and 1000 ml of 1.0 M NaCl in 1/30 M sodium phosphate

Amino acid compositions of GTX I and II

Amino acid	GTX I Nano-moles analyzed	Amino acid ratio	GTX II Nano-moles analyzed	Amino acid ratio
Lysine	7.10	4.10 (4)	6.61	4.01 (4)
Histidine	–	–	–	–
Arginine	5.09	2.94 (3)	4.33	2.62 (3)
Aspartic acid	3.57	2.06 (2)	3.39	2.05 (2)
Threonine	1.73	1.00 (1)	1.65	1.00 (1)
Serine	–	–	–	–
Glutamic acid	3.44	1.99 (2)	0.15	0.09 (0)
Proline	–	–	–	–
Hydroxyproline	5.20	3.01 (3)	4.46	2.70 (3)
Cysteine[a]	10.6	6.13 (6)	9.08	5.50 (6)
Glycine	–	–	–	–
Alanine	1.68	0.97 (1)	1.64	1.00 (1)
Valine	–	–	–	–
Methionine	–	–	1.01	0.61 (1)
Isoleucine	–	–	0.25	0.15 (0)
Tyrosine	–	–	–	–
Phenylalanine	–	–	–	–
Tryptophan[b]		–		–

[a] Determined as cysteic acid; [b] determined by UV absorption.



Effects of geographutoxin (GTX) I (2×10^{-7} M) and II (2×10^{-7} M) on the contractile response of the isolated mouse diaphragm to direct stimulation (0.1 Hz, 5 msec, supramaximal voltage). At △ and ↓, toxins were added and washed twice with fresh medium, respectively.