# Mucin (Qniumucin), a Glycoprotein from Jellyfish, and Determination of Its Main Chain Structure

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We extracted a novel glycoprotein, a member of the mucin family, from five species of jellyfish with high yields (1%−3% dry weight, 0.02%−0.1% wet weight) and determined its main chain structure and molecular mass. The glycoprotein contains unique tandem repeats of eight amino acids, of which two threonine residues are probably glycosylated by *N*-acetyl-D-galactosamine (GalNAc). We named this substance, which is common in jellyfish and similar to the human mucin MUC5AC, "qniumucin" and suggested the utilization of this compound as a new marine resource.

Recently, economic and social damage caused by the mass appearance of jellyfish[1] has frequently been reported. A typical incident involved damage to power plants located on the coasts, which draw seawater as coolant[2] (both thermal and nuclear power plants). Jellyfish accumulate in the tunnel, reducing water flow and causing shutdowns of the plant. Furthermore, in recent years, huge numbers of *Nemopilema nomurai*, one of the largest jellyfish in the world (diameter of 2 m, weight of 200 kg at maximum), were caught by net fishing in gulf areas of the Sea of Japan. Years of mass appearances of *Aurelia aurita*, which is the most abundant species found in all the oceans, have been investigated in the Black Sea area.[3]

The effect of global warming may increase the growth and survival rates of jellyfish, and artificial structures built on the coasts may provide a favorable environment for their polyps. Moreover, indiscriminate fishing reduces the number of fish that feed on plankton communities.[4] The removal of jellyfish has now become a necessary routine in gulf areas, ports, industrial facilities, and power plants along the coasts. As a result, a huge number of jellyfish bodies are collected and accumulate as waste. Although some trials have attempted to utilize jellyfish waste as fertilizer or food, no real solution has been found to cover the cost of removing jellyfish from the environment. Collagens can be extracted but are not very economically profitable because of their low prices.[5] Although various proteins found in jellyfish, such as collagens, have attracted the attention of researchers,[6] the existence of glyco-materials is poorly understood. In this report, we present the results of extraction of glyco-materials from jellyfish and the discovery of a new glycoprotein in the mucin family.

## Results and Discussion

We extracted glyco-materials from several types of jellyfish as described in the Experimental Section. The species examined were (a) *Nemopilema nomurai*, (b) *Aurelia aurita*, (c) *Chrysaora melanaster*, (d) *Rhopilema esculenta*, (a−d belonging to the class Scyphozoa), (e) *Chiropsalmus quadrigatus* (belonging to Cubozoa), and (f) *Spirocodon saltator* (belonging to Hydrozoa). After simple extraction and purification, we obtained a polymeric substance forming a gel in aqueous solution from all species.

A prominent and broad single peak of the glyco-materials was identified on an ion-exchange chromatogram together with several sharp peaks of proteins in every sample (see Figure S1 in Supporting Information), although for *S. saltator* (f), the broad main peak was not completely isolated from other components. We collected the fraction around this prominent peak in each case, and the initial yield was approximately 0.02%−0.1% wet weight, or 1%−3% dry weight.

The results of amino acid content analysis are shown in Table 1 for species a−e. The fractions of the contents were nearly a simple ratio, Val+Ile:Ala:Thr:Glu:Pro:GalNH$_2$ = 2:2:2:1:1:2, in all samples. Relatively small values for Val and Ile content were obtained because of slight resistance of Val−Val and Val−Ile bonds to hydrolysis. Amino acid sequence analysis for up to 30 residues from the N-terminal clearly revealed for a, b, and c a simple repeating unit of eight residues: Val-Val-Glu-$X_1$-$X_2$-Ala-Ala-Pro (where $X_1$ and $X_2$ were undetected). The sequencing results also indicated that a small fraction of substitution for Ile occurs in the second amino acid (Val), i.e., Val-Ile-Glu-$X_1$-$X_2$-Ala-Ala-Pro. Comparing this result with the amino acid contents shown in Table 1, both $X_1$ and $X_2$ should be threonine bonding to a sugar, probably *N*-acetyl-D-galactosamine (GalNAc). We performed $\beta$-elimination of the glycosylated threonine followed by EtSH addition[7,8] on the purified sample and compared the amino acid contents before and after the treatment. Threonine alone showed a significant decrease (1.9 → 0.6 normalized by alanine as 2.0), and the other components were unchanged. The change of components $X_1$ and $X_2$ to other unknown products was also detected in the sequence analysis (see Figures S2−S5, Supporting Information). Therefore, we concluded that this substance belongs to the mucin family in which some O-linked oligosaccharide side chains were attached via GalNAc to Ser and Thr of a single protein chain, i.e., (Val-Val(Ile)-Glu-Thr-(-GalNAc-?)-Thr(-GalNAc-?)-Ala−Ala-Pro)$_n$,[9−11] as shown in Figure 1. In this stage, there is a possibility that further glyco-chains are connected to GalNAc as indicated by "-?". We named this mucin "qniumucin". Although the amino acid sequence of the tandem repeat was apparently equal in a−c, one Val residue at the N-terminal appeared to be lost in species c. For d and e, sequence analyses showed an overlap of several components losing several residues from the N-terminal, although the existence of the same tandem repeat was indicated by both sequencing and amino acid content data. This heterogeneity did not depend on freshness, part of the body, or whether the jellyfish was dead or alive. Despite this slight heterogeneity, the tandem repeat of qniumucin started at the N-terminal without any additional domains. This characteristic is different from that of the majority of mucins discovered to date.[11−14]

Although the glyco-chains connect to two threonines as in other mucins, no serine was found in the tandem repeat. A similar tandem repeat composed of eight residues is also found in human mucin,

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**Table 1.** Results of Amino Acid Analysis and Molecular Mass Measurements of Qniumucin Obtained from Various Jellyfish Species

| species | *Nemopilema nomurai* | | | | | | *Aurelia aurita* | | *Chrysaora melanaster* | | *Rhopilema esculentae* | | | | *Chiropsalmus quadrigatus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| family/ order/ class | Rhizostomatidae/ Rhizostomeae/ Scyphozoa | | | | | | Ulmaridae/ Semaeostomeae/ Scyphozoa | | Pelagiidae/ Semaeostomeae/ Scyphozoa | | Rhizostomidae/ Rhizostomeae/ Scyphozoa | | | | Chirodropidae/ Cubomedusae/ Cubozoa | |
| place of catch (Japan) | off shore of Kyotango, Kyoto | | | | | | Hakata bay, Fukuoka | | off shore of Kyotango, Kyoto | | Hakata bay, Fukuoka | | | | Ishigaki Island, Okinawa | |
| part | ectoderm | | oral arm | | gonad | | whole | | whole | | mesoglea | | body fluid | | whole | |
| | mol% | ratio | mol% | ratio | mol % | ratio | mol% | ratio | mol % | ratio | mol % | ratio | mol % | ratio | mol % | ratio |
| Asp | 2.9 | | 1.9 | | 1.9 | | 1.2 | | 1.3 | | 3.6 | | 1.9 | | 1.0 | |
| Thr | 15.5 | 2 | 17.1 | 2 | 17.1 | 2 | 19.1 | 2 | 18.3 | 2 | 12.7 | 2 | 14.8 | 2 | 14.1 | 2 |
| Ser | 4.3 | | 2.3 | | 3.4 | | 0.6 | | 1.3 | | 2.9 | | 1.8 | | 2.9 | |
| Glu | 12.2 | 1 | 11.7 | 1 | 12.4 | 1 | 10.7 | 1 | 11.2 | 1 | 9.4 | 1 | 9.0 | 1 | 11.5 | 1 |
| Pro | 8.1 | 1 | 8.7 | 1 | 8.6 | 1 | 10.1 | 1 | 9.3 | 1 | 6.9 | 1 | 7.8 | 1 | 10.6 | 1 |
| Gly | 3.8 | | 2.2 | | 2.6 | | 0.8 | | 1.8 | | 3.7 | | 1.8 | | 1.9 | |
| Ala | 16.3 | 2 | 17.5 | 2 | 17.5 | 2 | 19.5 | 2 | 19.2 | 2 | 14.2 | 2 | 15.7 | 2 | 18.0 | 2 |
| Cys | 0.2 | | 0.1 | | 0.1 | | 0.00 | | 0.0 | | 0.0 | | 0.0 | | 0.0 | |
| Val | 10.9 | 2 | 11.7 | 2 | 12.1 | 2 | 15.2 | 2 | 12.4 | 2 | 9.5 | 2 | 10.6 | 2 | 9.9 | 2 |
| Ile | 2.4 | | 2.3 | | 2.40 | | 2.1 | | 3.5 | | 3.1 | | 2.9 | | 0.4 | |
| Met | 0.2 | | 0.1 | | 0.1 | | 0.1 | | 0.2 | | 0.5 | | 0.3 | | 0.8 | |
| Leu | 1.0 | | 0.6 | | 0.8 | | 0.4 | | 1.1 | | 3.2 | | 1.6 | | 0.8 | |
| Tyr | 0.5 | | 0.3 | | 0.4 | | 0.1 | | 0.3 | | 0.6 | | 0.3 | | 0.2 | |
| Phe | 0.6 | | 0.4 | | 0.5 | | 0.4 | | 0.4 | | 1.1 | | 0.6 | | 0.3 | |
| $GlcNH_2$ | 0.3 | | 0.4 | | 0.3 | | 0.1 | | 0.2 | | 0.9 | | 0.3 | | 0.1 | |
| $GalNH_2$ | 18.2 | 2 | 21.0 | 2 | 17.8 | 2 | 20.4 | 2 | 17.9 | 2 | 22.7 | >2 | 27.7 | >2 | 24.7 | >2 |
| Lys | 1.6 | | 1.1 | | 1.2 | | 0.8 | | 0.9 | | 2.6 | | 1.7 | | 1.9 | |
| His | 0.6 | | 0.3 | | 0.5 | | 0.0 | | 0.2 | | 0.7 | | 0.3 | | 0.2 | |
| Arg | 0.5 | | 0.3 | | 0.4 | | 0.2 | | 0.5 | | 1.5 | | 0.8 | | 0.5 | |
| yield (%)/ wet weight | 0.02 | | 0.064 | | 0.108 | | 0.05 | | 0.074 | | 0.036 | | | | 0.035 | |
| identified tandem repeat | VVETTAAP or VIETTAAP | | VVETTAAAP or VIETTAAP | | VVETTAAP or VIETTAAP | | VVETTAAP or VIETTAAP | | VVETTAAP or VIETTAAP | | probably VVETTAAP or VIETTAAP | | probably VVETTAAP or VIETTAAP | | probably VVETTAAAP or VIETTAAAP | |
| $M_w$ | 40 kDa | | 130 kDa | | | | 150 kDa | | 140 kDa | | 130 kDa | | | | | |
| $M_n$ | 20 kDa | | 60 kDa | | | | 80 kDa[a] | | 90 kDa | | 90 kDa | | | | | |

[a] Value is comparable to MM estimated by amino acid sequence analysis, 60 kDa.

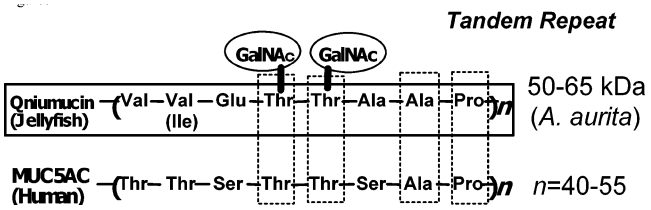


**Figure 1.** Structure of qniumucin with a tandem repeat of eight residues: (Val-Val(Ile)-Glu-Thr(-GalNAc)-Thr(-GalNAc)-Ala-Ala-Pro-)$_n$. The similar structure of human mucin, MUC5AC, is shown for reference. Boxes with dashed lines indicate four of the eight residues common to both mucins.

MUC5AC, of which the sequence is (Thr-Thr-Ser-Thr-Thr-Ser-Ala-Pro)$_n$.[11−13] The structure is provided in Figure 1 for reference. The four underlined amino acids (boxed in the figure) are identical in both mucins, forming the typical sequence of -Thr-Thr-$X_3$-Ala-Pro- ($X_3$ is Ala or Ser), where the Ala-Pro part is the periodic marker. Although MUC5AC is more flexible than quiumucin in promoting a wider variety of molecular recognition using glyco-chains, the physical properties of both mucins are similar because they form a gel solution when dissolved in water.

The retention times of the main peaks of the ion-exchange chromatograms (SEE S1, in Supporting Information) were different in each sample probably because of the difference in the molecular mass and/or the sugar components. A chromatographic analysis[15−17] of sugar components after hydrolysis was performed for qniumucin extracted from different types of jellyfish. As shown in Table 2, the results were rather complicated because the sugar components fluctuated depending on the species of jellyfishes, as well as each fraction of the ion-exchange chromatography. Table 2 shows the sugar components of the two consecutive fractions of the ion-exchange chromatography from the same sample of b to indicate the width of the fluctuation. These results illustrate that our purification is incomplete, especially for the contamination of glyco-materials (e.g., oligosaccharides), which have the potential to interact strongly with the sugar chains of mucin.

Other than GalNAc, relatively high amounts (10%−70% that of GalNAc) of arabinose (Ara) and galactose (Gal) were consistently detected in each sample. On the other hand, we found no sialic acids (Sia) in the components, which is in sharp contrast to the mucins from vertebrates. The question is the existence of extra sugar components, including Ara, that should originate from plants. In vertebrates and other animals, ordinary mucins are believed to be synthesized in the Golgi apparatus, and the sugar components are only of five types (Gal, Sia, GlcNAc, Fuc, and GalNAc). On the basis of the current results, we cannot rule out the possibility that sugar components other than GalNAc originated from impurities. Therefore, we conclude that the majority of the glycosylation sites should be Thr-GalNAc. A small number of GalNAc may bind the extra sugars detected by the component analysis or only adsorb glyco-materials such as oligosaccharides.

Since the total amount of extra sugars is small, we estimated the mass of the single repeating domain to be about 1200 Da, including the sugar part (=400 Da). Sugar and proteins roughly share the mass at a ratio of about 1:2. The number-average molecular weight ($M_n$) for the sample from b was estimated to be 50−65 kDa from the sequence measurements because the repeating counts of the tandem domain were estimated to be about 40−55 on average, assuming that the initial yield of Edman degradation is 50%−70%. We also performed size exclusion chromatography (SEC) as shown in Figure 2. The absolute value of molecular weight was determined for some fractions collected on the SEC (10.8−106 kDa) by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS). The calibration curve

**Table 2.** Results of Sugar Component Analysis of Qniumucins Obtained from Various Jellyfish Species

| species | *N. nomurai* | | | | | | *A. aurita* | | | | *C. melanaster* | | *R. esculenta* | | | | *C. quadrigatus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| part | ectoderm | | oral arm | | gonad | | whole (fraction 1[b]) | | whole (fraction 2[b]) | | whole | | mesoglea | | body fluid | | whole | |
| | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio | conc (pmol) | ratio |
| Gal | 71.20 | 0.5 | 25.59 | 0.3 | 25.51 | 0.5 | 25.10 | 0.6 | 39.6 | 0.6 | 9.70 | 0.5 | 85.59 | 0.7 | 55.07 | 0.5 | 14.98 | 0.1 |
| Man | 2.95 | 0.0 | 2.47 | 0.0 | 2.92 | 0.1 | 3.44 | 0.1 | 3.72 | 0.1 | 5.11 | 0.2 | 25.76 | 0.2 | 1.26 | 0.0 | 11.12 | 0.1 |
| Glc | 4.49 | 0.0 | 10.33 | 0.1 | 4.75 | 0.1 | 134.45 | 3.4 | 4.35 | 0.1 | 25.13 | 1.2 | 28.19 | 0.2 | 11.98 | 0.1 | 26.16 | 0.2 |
| Ara | 11.98 | 0.1 | 22.15 | 0.3 | 22.28 | 0.4 | 16.59 | 0.4 | 35.93 | 0.6 | 7.69 | 0.4 | 17.18 | 0.1 | 14.32 | 0.1 | 10.37 | 0.1 |
| Rib | 4.32 | 0.0 | 9.73 | 0.1 | 27.27 | 0.5 | 5.33 | 0.1 | 15.2 | 0.2 | 11.31 | 0.5 | 7.02 | 0.1 | ND | | ND | |
| Sia | ND[c] | | ND | | ND | | ND | | ND | | ND | | ND | | ND | 0.0 | ND | |
| Xyl | 3.32 | 0.0 | 4.0 | 0.1 | 2.5 | 0.0 | 6.27 | 0.2 | 3.04 | 0.0 | 9.49 | 0.5 | 4.17 | 0.0 | 4.94 | 0.0 | 2.87 | 0.0 |
| GlcNAc | 3.38 | 0.0 | 2.91 | 0.0 | 3.14 | 0.1 | ND | | 2.37 | 0.0 | ND | | 2.44 | 0.0 | ND | | 2.67 | 0.0 |
| Fuc | 6.76 | 0.1 | 5.55 | 0.1 | 16.71 | 0.3 | ND | | ND | | 21.10 | 1.0 | ND | | 3.5 | 0.0 | 4.92 | 0.0 |
| Rha | ND | | ND | | ND | | ND | | ND | | ND | | ND | | ND | | ND | |
| unknown[a] | 13.38 | 0.1 | 7.84 | 0.1 | 45.77 | 0.9 | 5.35 | 0.1 | 16.8 | 0.3 | 63.86 | 3.1 | ND | | ND | | 1.84 | 0.0 |
| GalNAc | 133.77 | 1.0 | 74.06 | 1.0 | 50.03 | 1.0 | 39.48 | 1.0 | 61.99 | 1.0 | 20.80 | 1.0 | 130.99 | 1.0 | 117.37 | 1.0 | 129.67 | 1.0 |
| total | 255.55 | | 164.63 | | 200.88 | | 236.01 | | 183 | | 174.19 | | 301.34 | | 208.44 | | 204.60 | |

[a] Unknown component always detected around the retention time of 29.8 min. [b] Next two fractions of ion-exchange chromatography from the identical sample. [c] ND: not detected.

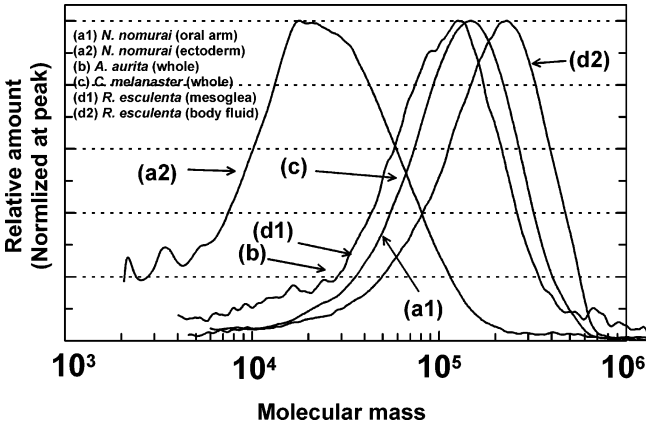


**Figure 2.** Molecular mass distributions obtained by size exclusion chromatography (SEC) of purified qniumucins extracted from whole body or various parts of four species of jellyfish: (a) *Nemopilema nomurai* (a1 from oral arm, a2 from ectoderm), (b) *Aurelia aurita*, (c) *Chrysaora melanaster*, and (d) *Rhopilema esculenta* (d1 from mesoglea, d2 from body fluid). Vertical axis is normalized by each peak intensity. Horizontal axis was calibrated using absolute values of molecular masses obtained through a MALDI-TOF experiment. Estimated $M_n$ and $M_w$ are summarized in Table 1.

for SEC was extrapolated above 106 kDa assuming a logarithmic dependence of the molecular weight on the retention time, which was confirmed by other polymers (pullulan) for the same column. The values of weight-average molecular weight ($M_w$) and $M_n$ evaluated by the SEC analyses are summarized at the bottom of Table 1. The estimated $M_n$ values of b obtained by the two methods, amino acid sequencing and SEC, seem equal within the experimental error.

As shown in Figure 2, the sample from the outer skin surface (ectoderm) of *Nemopilema nomurai* showed a low molecular weight ($M_w$ = 40 kDa, $M_n$ = 20 kDa), that is, 2%−30% of those of all the other samples from 1−5 of similar magnitudes. Since the mass distribution of the sample from the ectoderm showed a single peak, we can exclude the possibility of degradation of the original mucin in the extraction process and conclude that it was collected as it was secreted around the surface. Since a large amount of qniumucin was found in almost every organ of the jellyfish examined, it may be the source of mucus that sustains life. Jellyfish are known to secret mucus to clean their surface and to defend themselves against attacks from predators.[18] Further investigation is needed to clarify the function of qniumucin in jellyfish. Since a difference in species represented a difference in sugars but not in peptides, we can collectively name all mucins found in various jellyfish species with different sugar components as qniumucin.

Qniumucin is a highly polymerized mucin with a well-defined repeating domain and a widely distributed molecular mass. Similarity in the structure is noted for qniumucin and antifreeze proteins[19,20] (AFPs) extracted from insects, fish, and plants. One of the simplest structures of AFP to be artificially synthesized[21] is the polymer with a tandem repeat of Thr(GalNAc-Gal)-Ala. Accordingly, a similar antifreeze function is also expected for qniumucin from jellyfish.

The mass production of qniumucin is possible because a large number of jellyfish can be obtained as waste. Various applications are expected for this substance considering the present commercial use of gastric mucin from porcine stomachs and mucin from bovine submaxillary glands, substitution for human mucus, carriers for drug delivery, components of artificial extracellular matrices, antibiotic reagents, moisture retainers for cosmetic materials, and food additives.

As opposed to conventional mucins, however, qniumucin has a simple, well-defined structure with a tandem repeat of eight residues. At present, artificial formation of *O*-glycoside bonds is difficult, and the total synthesis of mucins has never been realized at an industry level, while many kinds of glycosyltransferase have been extracted from various biological systems. Therefore, qniumucin can be utilized as a starting material for the synthesis of tailor-made mucins. Some mucins have antibiotic functions, which can be improved with regard to effectiveness and selectivity via the modification of the glyco-chains. One example is the antibiotic function of human gastric mucin against *Helicobacter pylori* infection that is believed to originate from the existence of *O*-glycans that have terminal α-1,4-linked *N*-acetylglucosamine.[22]

In conclusion, a novel mucin, named qniumucin, was commonly found in a variety of species of jellyfish with markedly high yields. Qniumucin is similar to human mucin, MUC5AC. Qniumucin can be collected as a new marine resource suitable for the synthesis of tailor-made mucins.

## Experimental Section

**Catch and Storage of Jellyfish.** Jellyfish were caught at several points around the Japanese Islands as indicated in Table 1. Usually, samples were frozen and used after storage at −20 or −80 °C. Some samples (dead or alive) were directly transported to the laboratory at low temperature (4 °C) and used without freezing. All results in the present research showed no systematic dependence on the methods of storage and transportation.

**Extraction and Purification of Qniumucin.** The bodies of jellyfish were cut into small pieces and shaken in 0.2% NaCl aqueous solution

at 4 °C for 24 h. After centrifugation, a gel-like precipitate was obtained upon the addition of three times the volume of EtOH to the liquid. After standing overnight at 4 °C, the separated precipitate was dissolved again in $H_2O$ and salts were removed by dialysis. Finally, lyophilization of the solution yielded crude qniumucin.

Further purification was performed by ion-exchange chromatography as shown in Figure S1 in the Supporting Information. A TSKgel DEAE-5PW column (7.5 mm i.d. × 75 mm) from Tosoh Corporation was used with a gradient of 0% → 100% of B (0.5 M NaCl/10 mM $NaH_2PO_4$, pH 7) into A (10 mM $NaH_2PO_4$) in 0−20 min at a flow rate of 0.5 mL/min. We monitored two wavelengths for glycoprotein (215 nm) and proteins (275 nm).

**Structural Analysis.** Amino acid content was analyzed using a Hitachi L-8500A automatic amino acid analyzer after hydrolysis with 5.7 N HCl for 20 h at 110 °C. The amino acid sequences were analyzed using a Procise 494 HT from Applied Biosystems Inc. We also performed $\beta$-elimination followed by EtSH addition as described[7,8] and confirmed the sites of *O*-glycosylation by comparing amino acid content and sequence before and after treatment. Molecular mass distribution was measured by size exclusion chromatography using a TSKgel $G5000PW_{XL}$ column with 0.1 M $NH_4OAc$ aqueous solution as eluent. An aliquot of mucins was subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) on a Reflex (Bruker Daltonics, Bremen, Germany) in the linear mode using *trans*-indole-3-acrylic acid as matrix (Bruker Daltonics). The chromatographic analysis of sugar components was performed after hydrolysis using 4 M trifluoro acetic acid for 3 h at 100 °C. The sample was *N*-acetylated and labeled using a fluorescent label (ABEE) and then analyzed using a Honenpak C18 column (4.6 mm i.d. × 75 mm) from J-Oil Mills Inc., 0.2 M potassium borate buffer (pH 8.9) containing 7% $CH_3CN$, and a standard kit from J-Oil Mills Inc. containing 11 monosaccharides.[15,16]

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**Supporting Information Available:** Ion-exchange chromatograms of crude qniumucin from six species of jellyfish; the results of amino acid sequencing profiles, and chromatograms of purified qniumucin before and after $\beta$-elimination followed by EtSH addition. The materials are available free of charge via the Internet at http://pubs.acs.org.

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