a basic buffer system at pH 8.3[10] and in an acid buffer system at pH 4.5 according to Williams and Reisfeld[11]; 100 µl of the extract were used per polyacrylamide gel. Isoperoxidase bands were detected in the gel using benzidine as a hydrogen donor in 0.2 M acetate buffer pH 5.0. All experiments described above were repeated 3 times.

Results and discussion. It was found that the anodal isoperoxidase spectra of leaves of cv. Clara Butt and cv. Fantasy are similar. The same is true for leaves of cv. William Pitt and cv. Pitt's Parrot. However, the pattern of anodal isoperoxidases of tepals of cv. Clara Butt differs distinctly from cv. Fantasy (figure 1) and cv. William Pitt from cv. Pitt's Parrot (figure 2). It is interesting that in both parrot cultivars (Fantasy and Pitt's Parrot) the band designated in their mother cultivars (Clara Butt and William Pitt, respectively) by arrows (figures 1 and 2) was not detected. Additionally, a higher activity of peroxidase in tepals of cv. Fantasy than in the organ in cv. Clara Butt can generally be observed. The cathodal electrophoretic pattern of peroxidase in leaves and tepals of cv. cv. Clara Butt, Fantasy, William Pitt and Pitt's Parrot looks very similar. It is possible that the disappearance of the peroxidase band in the tepals of the parrot tulips is connected with their formation from mother non-parrot tulips.

It was found previously by Barber and Steward[12] that a specific electrophoretic pattern of soluble proteins and some enzymes exists in each organ of tulip such as roots, scales, leaves, vegetative axillary bud, tepals, anthers, and pistils. We concluded that these observations support the general view that differentiation and morphogenesis are accompanied by the formation of organ specific proteins and enzymes. Our results indicated that differentiation of laciniate tepals of parrot tulips is accompanied by a specific anodal isoperoxidase pattern in the tepals.

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Synthetic identification as a hexapeptide of α substance-I_B inducing sexual agglutination in Saccharomyces cerevisiae

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Summary. For the identification of a peptidyl principle inducing sexual agglutination in the yeast, 2 supposed hexapeptides (**1a**, **b**) were synthesized by the conventional method. The **1a** (H–Arg–Gly–Pro–Phe–Pro–Ile–OH) revealed complete identity with the natural peptide in TLC, MS and biological property on agglutination. The **1b** showed the sexual agglutinability in the same degree as **1a**, though distinct differences were observed in the chemical data. Both **1a** and **1b** had a strong bitter taste.

Recently, Sakurai et al.[3] isolated a peptidyl factor named α substance-I_B (abbreviated as I_B) from α type cells (H15 strain) of the heterothallic yeast, Saccharomyces cerevisiae, as one of the active principles which induce sexual agglutinability in the opposite *a* type cells (H22 strain)[4]. They attempted to determine the amino acid sequence in the peptide by mass spectrometry and postulated a tentative structure as H–Arg–Gly–Pro–Phe–Pro–Ile–OH (**1a**). In the beginning, however, an alternate structure, H–Arg–Pro–Gly–Phe–Pro–Ile–OH (**1b**) for the peptide could not be excluded due to the scarcity of samples to be used for the experiments. The present communication reports the syntheses of **1a** and **1b** by the conventional method and the identity of **1a** with the natural I_B.

Synthesis[5]. Boc–Pro–Ile–OBzl (**2**) was obtained as an oil from Boc–Pro–OH and H–Ile–OBzl TosOH by the mixed anhydride method[6], and **2** was converted to oily H–Pro–Ile–OBzl HCl (**3**) by the action of HCl in AcOEt. Oily Boc–Phe–Pro–Ile–OBzl (**4**) was prepared from Boc–Phe–OH and **3** by the mixed anhydride method. Removal of Boc group of **4** with HCl in AcOEt yielded oily H–tripeptide–OBzl HCl (**5**). Condensation of Boc–Gly–Pro–OH[7] with **5** gave oily Boc–Gly–Pro–Phe–Pro–Ile–OBzl (**7a**), and **7a** was converted to crystalline H–Gly–Pro–Phe–Pro–Ile–OBzl HCl (**8a**)[8]. Z–Arg(NO_2)–Gly–Pro–Phe–Pro–Ile–OBzl (**9a**) (73%, mp 99–103 °C, $[\alpha]_D^{20}$ −58° (DMF)) was obtained from Z–Arg(NO_2)–OH and **8a** by the mixed anhydride method. The **9a** dissolved in a mixture of AcOH–MeOH–H_2O was hydrogenated in the presence of Pd black. The filtrate was evaporated, and the residue (**1a**) was dissolved in water and lyophilyzed; yield of pure **1a** 2AcOH H_2O, 95%; mp 102–108 °C; $[\alpha]_D^{20}$ −88° (H_2O). Boc–Pro–Gly–OH (**6**) was obtained from Boc–Pro–OSu[9] and glycine. Condensation of **6** with **5** by the mixed anhydride method gave oily Boc–Pro–Gly–Phe–Pro–Ile–OBzl (**7b**), and **7b** was converted to H–Pro–Gly–Phe–Ile–OBzl HCl (**8b**) by the action of HCl in AcOEt. Z–Arg(NO_2)–Pro–Gly–Phe–Pro–Ile–OBzl (**9b**) prepared from Z–Arg(NO_2)–OH and **8b** contained minor by-product, and the pure **9b** (38%, mp 98–105 °C, $[\alpha]_D^{20}$ −34° (DMF)) was obtained by silica gel column chromatography (solvent, $CHCl_3$:MeOH:AcOH = 95:5:1). The

Thin-layer chromatography of natural and synthetic peptides

Solvent	Carrier	R_f I_B	**1a**	**1b**
n–BuOH–AcOH–H_2O (4:1:5, upper phase)	Silica gel	0.18	0.18	0.17
n–BuOH–pyridine–AcOH–H_2O (15:10:3:12)	Silica gel	0.59	0.59	0.57
n–BuOH–n–PrOH–0.2N AcOH (2:1:3, upper phase)	Silica gel	0.11	0.11	0.09
n–BuOH–n–PrOH–0.2N AcOH (2:1:3, upper phase)	Cellulose	0.58	0.58	0.63

1b was prepared from **9b** according to the same procedure as described for the preparation of **1a**; yield of pure **1b** 2AcOH H_2O, 95%; mp 107–112 °C, $[\alpha]_D^{20}$ −56° (H_2O).

Results and discussion. For the comparison of the synthetic peptides with I_B, TLC was performed at first using four different systems. As shown in the table, **1a** revealed identity with I_B. To compare mass spectra[10], the N-terminal arginine residues in **1a** and **1b** were converted to α-N-acetyl-pyrimidyl-ornithines and the C-terminus to methyl esters according to the procedure described in the previous paper[3]. The spectra of the derivatives from **1a** and **1b** (measured with a Hitachi RMU-6L, 70 eV, about 200 °C of sample temperature) showed quite a difference in their fragmentation patterns. The derivative from **1a** revealed ion peaks at m/e 777 (M^+), 585, 441, 344, 197 and 148, which were exactly the same as those observed for the corresponding derivative from I_B. Thus the identity of **1a** with I_B was verified unambiguously. The biological activities of **1a**, **1b** and I_B to induce agglutinability in *a* type cells (H22 strain) were assayed according to the method reported previously[4]. Contrary to our expectation, every compound tested showed the activity in almost the same degree at dosages of 2–20 ng/ml.

Ribadeau Dumas et al. elucidated the whole primary structure of bovine β-casein[11], which contained the sequence of –Arg(202)–Gly–Pro–Phe–Pro–Ile(207)–Ile–Val–OH(209) in the C-terminal portion. It is noteworthy that the part of –Arg(202)–Gly–Pro–Phe–Pro–Ile(207)– is just identical with I_B. Peptides having the structure of H–Arg–Gly–Pro–Pro–Phe–Ile–Val–OH (**10**)[12] and H–Gly–Pro–Phe–Pro–Val–Ile–OH (**11**)[13] were isolated as bitter principles from the enzymatic hydrolyzate of casein. We observed that the synthetic **1a** and **1b** had a strong bitter taste in almost the same threshold value at 0.13–0.25 mM (0.1–0.2 mg/ml). It is interesting that the peptide isolated as a sexual factor in the yeast has a very similar structure[14] to that of bitter principles.

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Collagen glycopeptides from the sea cucumber Stichopus japonicus

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Summary. Structures of several hydroxylysine-containing glycopeptides derived from the body wall of the sea cucumber have been determined. The glycosylated hydroxylysines were all the Y positions of Gly-X-Y triplets. They are thought to be distributed throughout the peptide chain, in contrast to vertebrate skin collagens. One glycopeptide demonstrated the existence of the sequence Gly-Gly-Hyp in invertebrates for the first time.

Glycoprotein nature of collagens has been established and many works have been carried out concerning the structure and biosynthesis of the carbohydrate units of the proteins[2–5]. In our previous works on invertebrate collagens[6,7], we observed the differences from vertebrates in the characteristics of the amino acid residues around the glycosylated sites. The present work was initiated to confirm whether such differences represent the reflection of the phylogenetic positions of the animals studied.

In this paper, we determined the structures of collagen glycopeptides from the body wall of the sea cucumber and compared them with those of vertebrate and invertebrate collagens.

Materials and methods. The body walls of Stichopus japonicus were defatted with acetone and minced in a blendor. The pronase P digestion was performed as described previously[7]. The digest was treated with cetylpyridinium chloride[8,9] and fractionated by gel-filtration through

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