activity in serum and TH activity in hypothalamus supports this view. Lamprecht et al.[12] also reported that, after 4 weeks of immobilization stress to rats, there was a significant increase in the activity of hypothalamus TH and in the activity of serum DBH. Interesting phenomenon in the present study is that TH activity in hypothalamus has a positive correlation with serum DBH activity only in young rats, but not in adult rats.

It may be concluded from the present results that serum DBH activity can be an index of peripheral sympathetic neurons during a long-term period and may be indirectly regulated by the central noradrenergic neurons through changes of peripheral sympathetic nerves, especially in blood vessels. Human serum DBH activity is determined by genetic factors, and therefore a great variation exists in men[1]. However, the present results indicate that a follow-up study of serum DBH activity with an individual patient may reveal the changes in the peripheral and central noradrenergic activity and may give an useful information as a diagnostic index in diseases such as essential hypertension in which the implication of the sympathetic nerves are suspected.

Linkages between chromophore and apoprotein in the biliverdin-protein of the scales of big blue parrotfish, Scarus gibbus Rüppell[1]

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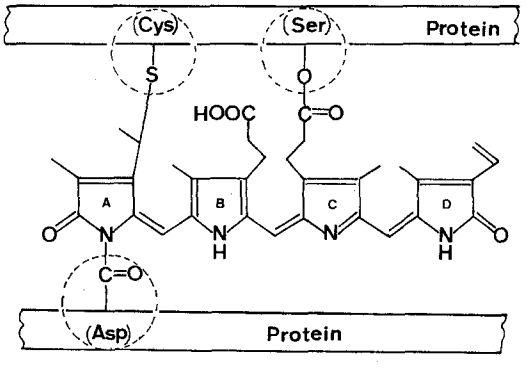
Summary. On the basis of the amino acid composition of the chromophore peptides, it can be assumed that Asp, Ser and $^1/_2$ Cys are located near the biliverdin chromophore. Experiments for splitting of the chromophore lead us to expect a thioether bond in the linkages between biliverdin and apoprotein.

Recently, Abolinš et al.[3,4] demonstrated that a blue chromoprotein isolated from the fins of Crenilabrus pavo C.V. was a biliverdin-protein. Subsequently it was shown by the present authors that the scale pigments of several species[5] of parrotfish, including S. gibbus and the skin pigment of the sculpin, Pseudoblennius percoides Günther[6], were also biliverdin-proteins, the fact suggesting a fairly wide occurrence of such chromoproteins in the epidermis of blue-coloured fishes. This investigation attempts to characterize the mode of linkages and the amino acids involved in the biliverdin-protein of S. gibbus[7].

The thin film in which the blue pigment is found was stripped off the scales, cut into small pieces and crushed with sea sand in a mortar. The pigment was extracted with 0.9% NaCl and fractionated with $(NH_4)_2SO_4$ between 0.2 and 0.4 saturation at pH 7.0 to collect the blue chromoprotein. Further purification by starch block electrophoresis[8] in 0.04 M phosphate buffer pH 6.8 afforded an electrophoretically homogeneous biliverdin-protein.

In order to find out which amino acid residues are involved in the linkage with biliverdin, the following procedure was applied. Approximately 50 mg of the lyophilized biliverdin-protein (P_0), of which amino acid composition was analyzed beforehand[9], were digested with 1 mg of pepsin[10] at pH 2.0 and 37 °C for 16 h. The digest obtained was submitted to Sephadex G-100 gel filtration (column 2.6 × 32 cm) with 0.1 N CH_3COOH as eluent. Fractions of 5 ml were collected and assayed for absorbance at 280 and 650 nm. The elution pattern gave 1 green and 8 colourless peaks. The green fractions were collected and lyophilized to obtain the biliverdin-peptide mixture (P_1), a part of which was subsequently analyzed for amino acid composition. P_1 was then digested with thermolysin[11] (enzyme to substrate ratio, 1:25) at pH 6.8 and 45 °C for 2 h. The digest was placed on a Sephadex G-10 column (1.6 × 70 cm) and eluted with 0.5 N CH_3COOH. The elution pattern gave one green and 5 colourless peaks. Green-coloured biliverdin-peptide mixture (P_2) was collected as above. A small portion of P_2 was assayed for amino acid composition. The remaining P_2 was further hydrolyzed with 2 N HCl at 100 °C for 2 h. The hydrolysate was subjected to paper chromatography with n-BuOH/CH_3COOH/water (4 : 1 : 5, by vol.) as solvent, resulting in the appearance of a green spot of biliverdin-



Presumed linkages between chromophore and apoprotein.

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10 A 3-times crystallized preparation of Worthington Biochemical Corp.
11 A crystalline preparation of Seikagaku Kogyo Co., Ltd.

Amino acid compositions of the 4 biliverdin compounds

	P_0	P_1	P_2	P_3
Asp	9.1	15.1	14.8	18.1
Thr	7.0	9.3	11.6	8.3
Ser	6.8	13.8	19.3	19.1
Glu	10.9	7.2	7.3	7.9
Pro	3.3	4.3	3.7	6.4
Gly	6.3	6.2	11.6	4.9
Ala	5.1	5.8	6.3	5.9
Val	5.6	4.0	0	0
Cys/2	3.0	7.9	6.9	20.9
Met	5.8	0	0	0
Ile	4.8	2.6	0	0
Leu	7.6	8.3	0	0
Tyr	2.3	0	0	0
Phe	4.7	4.0	4.1	0
Lys	8.5	5.6	4.8	3.1
His	4.5	3.5	6.9	5.4
Arg	4.7	2.4	2.7	0

Values are given as moles-% of amino acids found.

peptide mixture (P_3) together with 3 spots which were positive to Greig-Leaback reagent for peptide[12]. P_3 was extracted with 6 N HCl for amino acid analysis.

The amino acid compositions of the 4 biliverdin fractions were compared in molar percentage to total amount of amino acids found (see table). With the progressive shortening of the protein moiety of the pigment in the order P_0, P_1, P_2 and P_3, remarkable increases in molar percentage were found in aspartic acid, serine and half cystine. The rest of the amino acids showed no increasing tendencies, suggesting that the above 3 amino acid residues were bound to or located very near to the biliverdin.

The second approach to the elucidation of linkages was application of some specific splitting reactions. Neither acid acetone[13] nor boiling potassium hydroxide-methanol[3] could liberate the chromophore from the purified biliverdin-protein. However, on application of the acetic acid-silver sulphate method[14] employed in the splitting of haematohaeme from cytochrome c, the chromophore was released in a chloroform-soluble form. This was evidence that at least one of vinyl side-chains of biliverdin IX_α is linked to the sulfhydryl group of cysteine of the protein moiety through a thioether bond. In this connection, Köst-Reyes et al.[15] proved recently the occurrence of a thioether linkage in B-phycoerythrin mainly by the Edman degradation following pepsin digestion.

Finally, the nature of the linkages of the 4 pyrrole rings in the chromophore was examined by the chromic acid-dichromate degradation technique developed by Rüdiger[16]. On chromic acid oxidation at 20 °C, the biliverdin-protein yielded only half as much haematinic acid imide as that obtained at 100 °C, indicating that ring B or C is also linked to the protein moiety (see figure). Since the most probable partner is seryl residue on the basis of amino acid analysis, it may reasonably be deduced that the linkage should be an ester bond[17]. Furthermore, the fact that oxidation at 20 °C also yielded methyl vinyl maleimide indicates that at least one of the rings A and D is in a free state, and that even if an N-acyl bond[17] with aspartyl residue, the last possible partner, might exist, it should be located in the same pyrrole ring that is linked by the thioether bond. In addition, the dichromate degradation of the biliverdin-protein yielded pyrrole-dialdehyde, revealing that ring B is in an unbound state, so that the ester bond with seryl residue, if any, lies in ring C. In conclusion, a presumed mode of linkages between chromophore and apoprotein in the biliverdin-protein is given in the figure.

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Calcite growth under controlled diffusion[1]

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Summary. The growth of calcite was studied in a gelatin-gel medium under variable environmental conditions by 2 different methods. The results suggest that the organic matrix, the temperature, the diffusion fluctuation depending on ionic concentrations, and the presence of additives exert a fine control on the evolution of single crystals, polycrystalline aggregates, and highly structured concretions of calcite.

The environmental conditions that trigger biological calcification have been of considerable interest. In biomineralization, the organic matrix is considered as the prime substrate on which the mineral phase develops[4–6]. Recently, we reported the in vitro growth of organized calcite concretions in a gelatin-gel medium by slow diffusion of ions[7]. In view of these findings, the formation of these concretions in nature could be regarded as a chemical event, induced by some fibrous element and further dictated by diffusion kinetics and environmental conditions. This report describes the effect of the temperature, the ionic concentrations, and the presence of formaldehyde on the growth behavior of calcite crystals as studied by 2 different laboratory methods of crystal growth in gelatin-gel.

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