of 0.1 M NaCl is sufficient to promote dissociation of the hemoglobin tetramer into dimers[13,14]. Rosenmeyer and Huehns have suggested that of the 2 types of dimer possible, $\alpha_1\beta_1$ and $\alpha_1\beta_2$ that dissociation to the $\alpha_1\beta_1$ dimer is more likely since reaction of the β-93 sulfhydryl which lies near the $\alpha_1\beta_2$ interface should lead to formation of the $\alpha_1\beta_1$ dimer. However, it is in the $\alpha_1\beta_2$ dimer that the masked sulfhydryls α-104 and β-112 are exposed to further reactions with PMB. The rate expression for the reaction of hemoglobin with PMB involves a term with $[Hb]^{1/2}$. It can be shown that this requires participation of the dimer in the slow step. Participation of monomers would require a term involving $[Hb]^{1/4}$. Thus the $\alpha_1\beta_2$ dimer is probably the reactive dimer.

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Quenching of intrinsic fluorescence accompanies the activation of prococoonase[1]

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Summary. The intrinsic fluorescence of prococoonase from *Bombyx mori* is largely quenched upon its activation. The rates of fluorescence quenching and enzyme activation are equal, indicating that both reflect the same process.

Cocoonase is a proteolytic enzyme used by some silkworms to digest the protein sericin of the cocoons, hence facilitating their escape[2-4]. It is synthesized in the galeae first as prococoonase, a zymogen which can be activated by bovine trypsin, proteolytic enzymes in the moulting fluid and cocoonase itself, in the course of which a portion of the polypeptide chain is cleaved from prococoonase[4-6]. In this report it is shown that activation of prococoonase from *Bombyx mori* is accompanied by a large quenching of intrinsic fluorescence.

Materials and methods. Prococoonase was prepared by a method adapted from Berger et al.[4]. Galeae from 60 *B. mori* moths on the last day before emergence from cocoons where collected in 0.1 M potassium phosphate buffer pH 6.6 containing 10 mM sodium cyanide (0.8 ml). After grinding in an all-glass homogenizer, the mixture was centrifuged at 12,000 × g for 10 min. The supernatant was made 90% saturated by addition of solid ammonium sulphate. Most of the proenzyme remains in the supernatant which, after dialysis and concentration, was passed through a column of Sephadex G-75 (1 × 50 cm) and eluted with the same buffer. Only a single peak is obtained from the column, and it was shown to be homogeneous on dodecylsulphate-polyacrylamide gel electrophoresis.

The proenzyme (1.5 mg/ml, 0.04 ml) was activated by addition of trypsin solution (0.2 mg/ml in 0.1 M Tris-HCl pH 8.0, 40 mM KCl, 10 mM KCN, 0.16 ml). Enzyme activity was measured by adding the activated mixture (0.02 ml) to a solution of 0.1 M Tris-HCl pH 8.0, 40 mM KCl, 10 mM KCN, 0.5-2 mM benzoylarginine ethyl ester (BAEE) (0.98 ml). Hydrolysis was followed by spectrophotometry at 258 nm (Beckman DU monochromator and

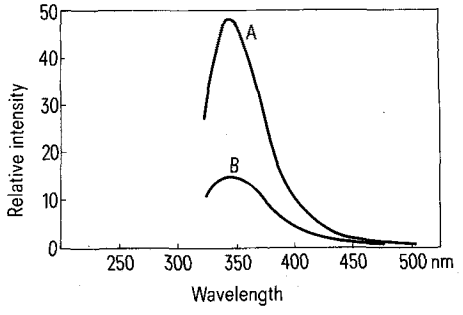

Fig. 1. Fluorescence spectra of (A) prococoonase and (B) the mixture of cocoonase and the activation peptide. The proenzyme was activated as indicated in 'Materials and methods'.

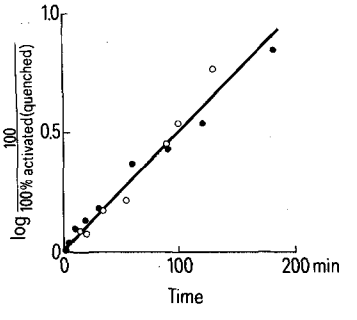

Fig. 2. The plots of log 100/(100-% activated enzyme) against time (○) and of log 100/(100-% maximum quenching) against time (●).

Gilford 2000 photometer); $\Delta\varepsilon$ between benzoylarginine and BAEE = 600 M^{-1} cm^{-1}. Fluorescence was measured by Aminco-Bowman spectrofluorometer. The contribution from trypsin to the observed fluorescence was negligible under the experimental conditions used.

Results and discussion. Prococoonase obtained from *B. mori* was found to be homogeneous with a mol. wt of 27,000 on dodecylsulphate-polyacrylamide gel electrophoresis, and 32,000 on gel filtration. Upon activation by trypsin, 2 fragments are formed, which correspond to mol. wt of 21,000 and 10,000 on gel electrophoresis. The larger fragment is presumably cocoonase, for which a mol. wt of 20,000 has been previously reported[7]. With BAEE as substrate, the activated enzyme was found to have k_{cat} of 22 s^{-1} and K_m of 1.0 mM at pH 7.8, 24 °C. These values may be compared with k_{cat} of 36 s^{-1} and K_m of 0.057 mM reported for cocoonase from *Antheraea* pernyi[3], and k_{cat} of 51 s^{-1} and K_m of 0.048 mM for cocoonase from *A. polyphemus*[5].

The intrinsic fluorescence spectrum of prococoonase, with excitation at 290 nm, is as shown in curve A of figure 1. The spectrum indicates main contribution from tryptophan residues in the protein. Upon activation by trypsin, the spectrum of the resulting mixture of cocoonase and the activation peptide is as shown in curve B of figure 1, with the same λ_{max} of 342 nm, but a much reduced intensity. The quenching of fluorescence, to a total extent of 70% on complete activation, indicates that the tryptophan residues of prococoonase are in a different environment from those of the activated products. In contrast, inspite of extensive homologies between the cocoonases and trypsin[6], the fluorescence of trypsinogen is little changed on activation to trypsin[8]. Since there are only 2-3 tryptophan residues in prococoonase from various sources, none of which is lost from the enzyme on activation[6], the large quenching of fluorescence may reflect changes only in the microenvironment of these residues, and not necessarily protein conformational changes. Addition of 1 mM BAEE did not produce any change in the fluorescence spectrum of activated cocoonase.

The rate of activation of prococoonase by trypsin, as followed by activity assay with BAEE as substrate, is first order as shown in figure 2, in agreement with the result of Felsted et al.[5]. The rate of fluorescence quenching at 340 nm is also first order with respect to cocoonase, with the kinetic constant identical to that of enzyme activation for at least 2 half-lives (figures 2). It can be concluded that the change in environment of the fluophores occurs simultaneously with the formation of the active enzyme. The quenching of fluorescence can therefore be conveniently utilized to study the process of activation of this enzyme, especially when this is too rapid to follow accurately by activity assay.

1 This work was supported by a grant from the National Research Council of Thailand.
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Inhibition of prostaglandin-induced cyclic AMP accumulation in the rat anterior pituitary by alrestatin

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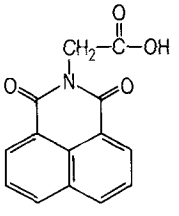
Summary. Alrestatin at 25-1 × 10^{-4} M inhibited the accumulation of cyclic AMP induced by prostaglandin E_2, but not theophylline, in the rat anterior pituitary in vitro. Somatostatin, at lower concentrations, inhibited both; maximal inhibition of the prostaglandin effect was greater with alrestatin. As cyclic AMP is considered to be a mediator in induced-hormonal release, it appears from the present findings that alrestatin may be of potential use in altering hormonal release.

Cyclic AMP appears to be a mediator in prostaglandin-induced hormonal release. For growth hormone such a release has been observed in the rat anterior pituitary in vitro[2-5]. In vivo the plasma growth hormone levels are increased by administration of prostaglandin[6]. Prostaglandins and the cyclic nucleotide phosphodiesterase inhibitor theophylline increase both the accumulation of cyclic AMP and the release of growth hormone in the anterior pituitary in vitro[5,7,8]. The prostaglandin- and the theophylline-induced increases in cyclic AMP accumulation in the anterior pituitary in vitro are inhibited by somatostatin, the growth-hormone release-inhibiting hormone[7,8], as is the accompanying immunoreactive growth hormone and thyrotropin release[8]. Somatostatin also antagonizes the prostaglandin-, theophylline- and N^6-monobutyryl cyclic AMP-induced releases of immunoreactive growth hormone and thyrotropin from rat anterior pituitary cells in monolayer culture[9].

As with somatostatin, various of its analogs have been shown to be effective inhibitors of the prostaglandin-induced cyclic AMP accumulation in the anterior pituitary in vitro[10]. In the present studies alrestatin (figure), an aldose reductase inhibitor[11], has been found to antagonize the prostaglandin-induced increase in cyclic AMP in this system.



Materials and methods. The method employed in the determination of the accumulation of cyclic AMP in the anterior pituitary was based upon that reported previously[12,13] and was carried out as previously described[10,14]. The tissues were incubated, with shaking, for 60 min at 37 °C in an atmosphere of 5% CO_2 - 95% O_2 in 1.0 ml of Krebs Ringer bicarbonate buffer containing 11 mM D-glucose. The incu-