

Complex formation between a formyl peptide and 24p3 protein with a blocked *N*-terminus of pyroglutamate

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We have purified 24p3 protein from mouse uterine fluid (*Biochem. J.* **316**, 545–550, 1996). It is a 25.8-kDa glycoprotein with a *N*-blocked terminus. This work demonstrated the *N*-blocked residue to be pyroglutamate, supporting the post-translational cleavage site at Ala-Gln in the precursor protein to generate a putative protein of 180 amino acid residues. Consequently, the two cysteines, Cys⁷⁸ and Cys¹⁷⁷, and the two tryptophans, Trp³¹ and Trp⁸¹, are assigned along the polypeptide chain. No free thiol group was detected in the protein. The presence of formyl-Met-Leu-Phe in the protein solution causes a considerable decrease in the protein fluorescence due to Trp³¹ and Trp⁸¹. Analysis of the fluorescence data supports the idea that the protein can be complexed with the formyl peptide. The association constant for the complex formation is $(4.8 \pm 0.29) \times 10^5 \text{ M}^{-1}$ at pH 7.4. © Munksgaard 1997.

Key words: fluorescence; formyl peptide; 24p3 protein; pyroglutamate; pyroglutamate amino peptidase

Rodents have been used as important experimental animals for studying the reproductive biology of mammals. The accumulation of uterine luminal fluid (ULF) in the proestrus phase of rodent reproductive cycle is well known. The phenomenon also occurs in the immature female rodent stimulated by estrogen or its analogues. Thus, establishing the structure and function of protein components in ULF becomes important in order to understand their roles in the growth and development of the reproductive tract(s) concerned. In this regard, we have demonstrated recently a glycoprotein derived from 24p3 mRNA in mouse ULF (1). 24p3 cDNA was originally cloned from SV40-infected mouse kidney primary culture cells (2). The protein derived from this gene (hereafter referred to as 24p3 protein) was found also in lipopolysaccharide-stimulated mouse PU5.1.8 macrophage cells (3) and bFGF-stimulated 3T3 cells (4). The results of Liu and Nilson-Hamilton reveal 24p3 protein to be an acute phase protein (5). Its structure and function have not yet been reported. Here we report the complex formation of formyl-Met-Leu-Phe and 24p3 protein that was demonstrated to have a blocked *N*-terminus of pyroglutamate.

EXPERIMENTAL PROCEDURES

Materials. Outbred ICR mice were purchased from Charles River Laboratory (Wilmington, MA) and

were bred in the animal center at the College of Medicine, National Taiwan University. Animals were treated following institutional guidelines for the care and use of experimental animals. Diethylstilbesterol (DES), 5,5'-dithiobis(2-nitrobenzoic acid) (Ellmen's reagent) and formyl-Met-Leu-Phe (formyl peptide) were from Sigma (St. Louis, MO, USA). Calf-liver pyroglutamate aminopeptidase (E.C.3.4.19.3) was from Boehringer Mannheim G.m.b.H. (Germany).

Preparation of 24p3 protein. Mice were housed under controlled lighting (14 h light, 10 h dark) at 21–22 °C and provided with water and National Institutes of Health 31 laboratory mouse chow *ad libitum*. DES dissolved in corn oil was administered subcutaneously to female mice (3 wk old) with a daily dose of 100 ng/g body weight for three consecutive days. Subsequently, the animals were killed and ULF was collected. 24p3 Protein was purified from mouse ULF according to our previous procedure (1).

Protein analysis. 24p3 Protein was digested with pyroglutamate aminopeptidase by a modified method of Henze *et al.* (6). After the protein (20 µg) in 100 µL of sodium phosphate (pH 7.0) containing 0.1 mM EDTA was digested with the enzyme (2 µg) at 4 °C for 18 h, the solution was incubated further at 25 °C for 4 h. The solution was subjected to HPLC

thiol group that is reactive to associate covalently with gelatinase. However, the situation would not happen to 24p3 protein, since it is devoid of free thiol group.

The interaction of 24p3 protein and formyl peptide

Figure 2 displays the emission spectra of 24p3 protein under several conditions. Excitation was at 295 nm to ensure the fluorescence due to Trp³¹ and Trp⁸¹. The native protein in phosphate-buffered saline at pH 7.4 (Fig. 2, curve I) exhibits a peak at 332 nm. The peak shifts to 350 nm, and the intensity increases greatly when the protein is in 6.0 M guanidinium hydrochloride (Fig. 2, curve II). Further reduction of the unfolded protein with 1,4-dithiothreitol shifts the peak to 352 nm (Fig. 2, curve III). Apparently, the two tryptophan residues are restricted into a configuration that differs from that of free tryptophan in aqueous solution.

Human NGAL is a glycoprotein with the binding capacity for formyl-Met-Leu-Phe (12). It raises the question of whether 24p3 protein can be complexed with the formyl peptide. Figure 3 gives the fluorescence emission spectrum of 24p3 protein at a concentration of 0.8 μ M in the presence of 80 mM formyl peptide. Relative to the protein fluorescence, the formyl peptide itself shows very weak fluorescence intensity. The formyl peptide in the protein solution does not shift the emission peak of protein but effects a considerable decrease in the protein intrinsic fluorescence. Fitting the data of F_{295}^{332} obtained from adding formyl peptide to the protein solution with eqn. (1) shows a linear curve in the

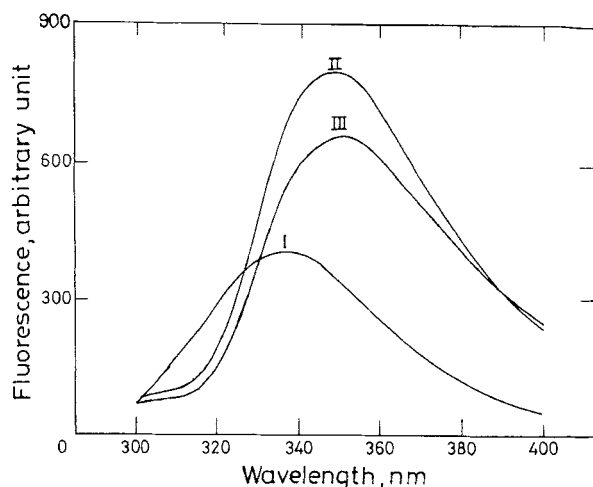


FIGURE 2

Fluorescence emission spectra of 24p3 protein. The emission spectra were scanned with excitation wavelength at 295 nm. The protein was at 1.0 μ M in phosphate-buffered saline at pH 7.4 (curve I) or 6.0 M guanidinium hydrochloride in the absence (curve II) and in the presence of 10 mM 1,4-dithiothreitol (curve III).

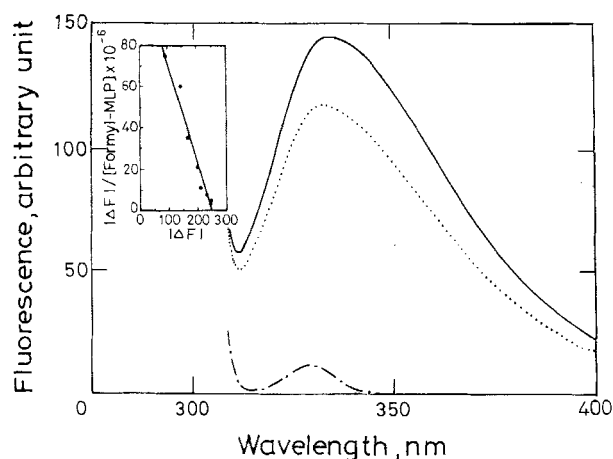


FIGURE 3

Effect of formyl-Met-Leu-Phe on the fluorescence of 24p3 protein in phosphate-buffered saline at pH 7.4. The emission spectra were scanned with excitation wavelength at 295 nm. The protein was at 0.8 μ M alone (—) and in the presence of 80 mM formyl peptide (---). The fluorescence of 80 mM formyl peptide alone was very weak (···). The modified Scatchard plot for the binding of formyl peptide to 24p3 protein is given in the inset. The data obtained from adding formyl peptide to the protein solution were analyzed by using eqn. (1) with linear-regression fitting. The correlation coefficient was calculated to be more than 0.96.

modified Scatchard plot (Fig. 3, inset), manifesting that the protein has one singular type of formyl peptide-binding site. The association constant for the affinity site was estimated to be $(4.8 \pm 0.29) \times 10^5 \text{ M}^{-1}$, which is comparable with that of the complex formed by formyl peptide and human NGAL (12).

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