A PEPTIDE, CONTAINING THE REACTIVE SULFHYDRYL GROUP OF Ca^{++} ACTIVATED ATPase IN H-MEROMYOSIN

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1. Introduction

After labelling with ^{14}C NEM Turba and associates [1] previously demonstrated a cysteine containing peptide in the tryptic fingerprint that was definitively correlated with the activity of Ca^{++} activated ATPase in H-Meromyosin.

We undertook the purification and separation of the ^{14}C NEM labelled peptides. The amino acid analysis of peptide "4" of Turba et al. [1] was found to be in fair agreement with the sequence Cys-Gly-Asn-Val-Leu-Glu-Gly-Ile-Arg-(Ile-Cys-Arg) communicated [2] after investigations with other labelling procedures.

2. Materials and methods

H-Meromyosin was prepared according to Gröschel-Stewart and Turba [3]. ^{14}C NEM (specific activity 2.9 $\mu C/\mu M$, Schwarz Bioresearch Inc., USA) was added in quantities equimolar to the sulfhydryl groups to protein solutions of 0.4–0.6 g protein (Kjeldahl) in 30–40 ml 5 M guanidine. After exhaustive dialysis and lyophilization, the protein was reduced and carboxymethylated according to the method of Crestfield, Stein and Moore [4]. About 1 g of the tryptic digest [3] was filtered through a Sephadex G-25 column (fig. 1). Fractions II, III, and $\frac{1}{2}$ IV were pooled and lyophilized. The lyophilized material was dissolved and layered onto a SE-Sephadex C-25 column. (fig. 2). Subsequently, fraction II from the SE-Sephadex chromatography was lyophilized, dissolved and layered onto a DEAE-Sephadex A-25 column. (fig. 3). The lyophilized fraction III from DEAE Sephadex chromatography was desalted on a Sephadex G-15 column (18 X 1825 mm), equilibrated with H_2O/NH_3 pH 9.0. The desalted fractions were then subjected to separation on silica gel or paper electrophoresis, and by ascending chromatography in n-butanol-pyridine-acetic acid-H_2O 30:20:6:24 (v:v).

The labelled peptide (arrow) was finally analysed. (fig. 4).

Amino Acid Analysis:

	μMole
Arg	0.152
Asp	0.148
Ser	trace
Glu	0.168
Gly	0.301
Ala	0.005
Val	0.162
Ile	0.142
Leu	0.170

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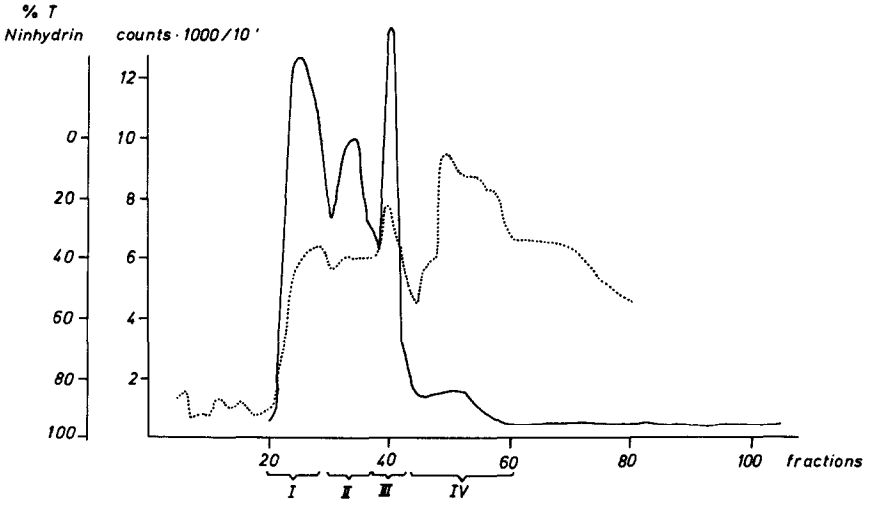

Fig. 1. Sephadex G-25 Gel filtration. A column of 22 X 2850 mm was equilibrated with 0.001 M Na_2CO_3. About 1 g of the tryptic peptides was layered onto the column. Ninhydrin . . . . . Radioactivity ——— .

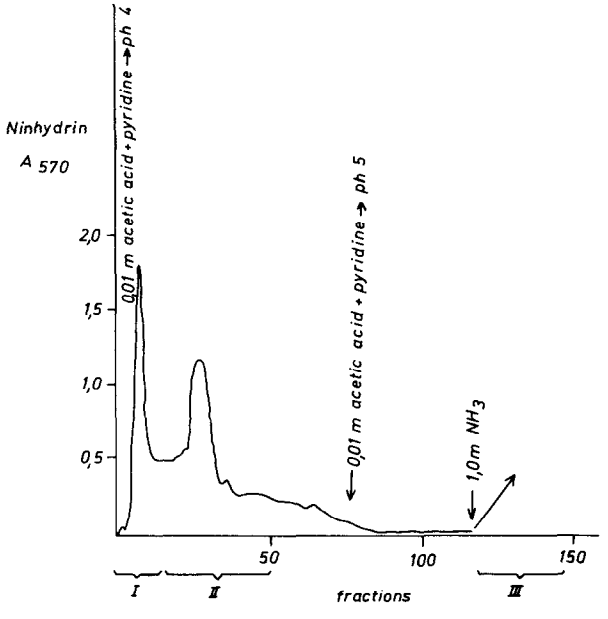

Fig. 2. SE-Sephadex-C-25 Chromatography. A column of 25 X 150 mm was equilibrated with 0.01 M acetic acid/pyridine pH 3.7. About 600 mg of fractions II, III and $\frac{1}{2}$ IV from Sephadex G-25 gelfiltration were dissolved in the starting buffer and layered onto the column. Anodic and most of the neutral peptides were eluted before the pH was raised to 5. Finally the column was eluted with 1.0 M NH_3 for the cathodic peptides. Fraction II was lyophilized.

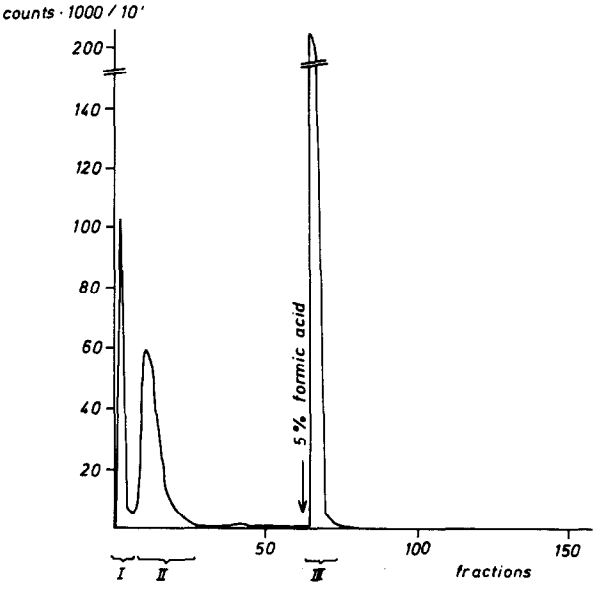

Fig. 3. DEAE Sephadex A-25 Chromatography. A column of 12 X 170 mm was equilibrated with 0.1 M pyridine/acetic acid pH 6.0. The lyophilized fraction II from SE-Sephadex chromatography was dissolved in the pH 6.0 buffer and layered onto the column. The column was washed with the pH 6.0 buffer until no more ninhydrin positive material could be eluted. All of the neutral peptides were eluted. The anodic peptides were then eluted with 5% formic acid. Fraction III was lyophilized.

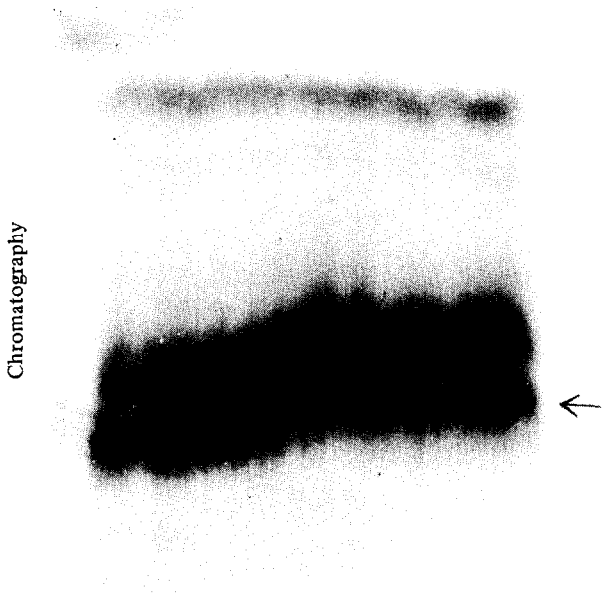

Fig. 4. Autoradiography from Paper Chromatogram of Band II c from Sephadex G-15 Gelfiltration. Whatman 3 MM paper. Ascending chromatography in n-butanol-pyridine-acetic acid-H_2O 30:20:6:24 (v:v) for 21 hr. An autoradiograph developed for $3\frac{1}{2}$ weeks.

3. Discussion

An amino acid analysis on a previous state of purification (i.e. before silica gel or paper electrophoresis) provided evidence for a double value of Ile and Arg. The tryptic NEM labelled peptide, the analysis of which is shown above can be separated only incompletely from a second peptide, identified as S_1^5 (W.W.Kielley, personal communication), in all chromatographic procedures, except the final paper chromatography.

The amino acid analysis of peptide "4" provides further support for the S_2 peptide, partly described by Yamashita et al. [6] and analysed by Kimura and Kielley [7]. A conflict, however, remains in respect to the function or meaning of the sulfhydryl groups S_1 and S_2, and peptide "1" of Turba et al. [8].

As originally found, a complete decrease of EDTA ATPase activity was correlated with the alkylation of S_1 by NEM in myosin [9], and with the labelling of peptide "1" in H-Meromyosin [8]. The activating effect on Ca^{++} ATPase was related to the S_1 sulfhydryl, however, and not to the peptide "1" SH-group.

Perry and Cotterill [10] recently demonstrated the differential effects of varying KCl concentrations on the Ca^{++} ATPase activity in myosin, and, especially, in HMM. KCl concentrations of 0.5 M, as used in the investigations on S_1 and S_2 were shown to have a strong activating effect on the Ca^{++} ATPase. As the concentrations of KCl used in the investigations of Turba et al. were smaller (up to 0.2 M KCl) a different pattern of ATPase activation or inhibition resulted. From this one may conclude that a different reactivity of the sulfhydryl groups might have resulted. Therefore, labelling of peptide "1", different from peptide "4" and from the S_1 and S_2 peptides was found responsible for the decrease of EDTA ATPase activity.

In view of more recent investigations [11] and on possible allosteric transitions in HMM and myosin [12] the function of the SH regions should be reinvestigated in various ionic environments to find out, whether the SH group of peptide "1" is only functional at, say, lower ionic strength.

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