# COOPERARIVE PARTICIPATION OF TWO PEPTIDES FROM β-CASEIN IN LEUKOCYTE CHEMOTAXIS

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Received 26 June 1976
Revised version 27 June 1976

## 1. Introduction

Studies on how leukocytes recognize chemotactic factors and on where the recognizing locus of a chemotactic factor is, have become an attractive field for the membrane biochemist and the protein chemist. Bokisch et al. [1] identified a fragment of the third component of human complement (C3a), and Ward and Newman [2] identified a fragment of the fifth component of complement (C5a) as chemotactic factor. Fibrinopeptide B [2], which is produced in fibrinogen-fibrin conversion by thrombin, and kallikrein [4] have also the chemotactic activity for human leukocytes. Wissler et al. [5] demonstrated that the chemotactic activity for neutrophiles displayed by the coexistance of classical anaphylatoxin and cocytotaxin. The chemotactic activity of various proteins for leukocyte has been extensively studied by Wilkinson and McKay [6] in relation to the structure of the protein molecule, and they suggested that the chemotactic recognition of structurally altered proteins by neutrophiles depends on hydrophobic bonding between non-polar groups on the protein and those on the cell surface.

The present paper deals with the recognizing locus in the β-casein molecule for leukocyte chemotaxis.

## 2. Materials and methods

Guinea pig polymorphonuclear leukocytes were obtained intraperitoneally by the method of Kakinuma [7], using 1% sodium caseinate as an irritant. The peritoneal exudate cells were washed three times with phosphate buffered saline (PBS) and rewashed four times with Hanks' solution. The chemotactic activity of casein for leukocyte was determined by measuring the chemotactic migration of leukocytes through a Millipore filter in the presence of casein, according to the method of Zigmond and Hirsh [8]. The distance between the surface of a Millipore filter and the front of cells advancing into a Millipore filter by attractant was measured in a fixed time by direct microscopic observation. Whole casein and β-casein were isolated from fresh milk of individual Holstein cows by the method of Thompson and Kiddy [9] and were used as an attractant for leukocyte. β-Casein (1 g) was cleaved by cyanogen bromide (0.8 g) in 0.1 N HCl for 24 h at room temperature according to the method of Gross and Witkop [10]. The mixture of peptides obtained by degradation of β-casein with cyanogen bromide was passed through Sephadex G-50 column equilibrated with 30% acetic acid, and the concentrations of peptides eluted were determined by measuring the absorbance at 280 nm. On the chromatographic pattern, the protein fractions corresponding to two peaks with a shoulder have the chemotactic activity. In order to purify further, the protein fractions with the chemotactic activity were passed through a CM-cellulose column equilibrated with 20 mM ammonium acetate and then eluted with a linear gradient (0–100 mM NaCl). The two protein fractions were obtained in pure form and were named peptide A and peptide B. Disc electrophoresis was carried out with 7.5% polyacrylamide gel containing 4 M urea (pH 9.5). The amino acid compositions of the peptides were determined with an amino acid analyzer model JLC-5AH after hydrolysis at 110°C for 22 h in 6 N HCl.

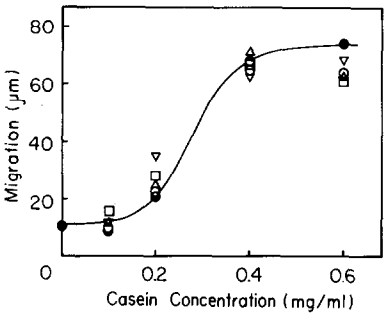


Fig.1. Plotting of the chemotactic activity of casein for guinea pig leukocytes against concentration of casein: •; native casein, △; casein denatured with 1.7 M urea, ○; casein denatured with 6.6 M urea, ▽; casein denatured by alkali (pH 12), □; casein denatured by heat (100°C for 15 min).

## 3. Results and discussion

In order to clarify whether the higher order structure of the casein molecule plays an important role in leukocyte chemotaxis or not, the leukocyte chemotaxis was measured with native casein and denatured casein as an attractant. Figure 1 shows plotting of leukocyte chemotactic activity against amount of whole casein. In native whole casein, the chemotactic activity enhances with increasing the casein concentration and tends to approach a constant level. The similar phenomena were observed for alkali-denatured, heat-denatured and urea-denatured whole caseins. This indicates that the characteristics of casein as an attractant do not associate with the higher order structure of the casein molecule.

The peptides A and B which were obtained by the degradation of β-casein with cyanogen bromide and followed by chromatography were subjected to disc electrophoretic analysis. A single sharp band was observed for the peptide A (tube A), the peptide B (tube B) and β-casein (tube C), which are shown in fig.2, respectively.

The chemotactic activity was measured with the mixture of peptides obtained by degradation of β-casein with cyanogen bromide. The chemotactic activity thus measured was almost the same degree as when β-casein was used as an attractant. On the other hand, the chemotactic activity was scarcely observed when adding each peptide in a pure form. This suggests that more than two peptides as attractants

Fig.2. Disc electrophoretic patterns of the peptide A (tube A), the peptide B (tube B) and β-casein (tube C).

were necessary to exhibit the leukocyte chemotaxis.

Figure 3 shows the cooperative participation of two peptides, the peptides A and B, in the leukocyte chemotaxis. On the left side panel of this figure, curves 'a' and 'b' represent plotting of migration distance of leukocyte against peptide B concentration in the presence and the absence of the peptide A, respectively. Presence of the peptide B scarcely enhances the chemotactic activity, while coexistance of the peptide A and the peptide B markedly enhances the chemotactic activity. The maximum value of migration distance in the coexistance of the peptides A and B was about two-thirds of that in β-casein. On the right side panel, curve 'a' shows a similar plotting of the chemotactic activity in the presence of 0.064 mg/ml

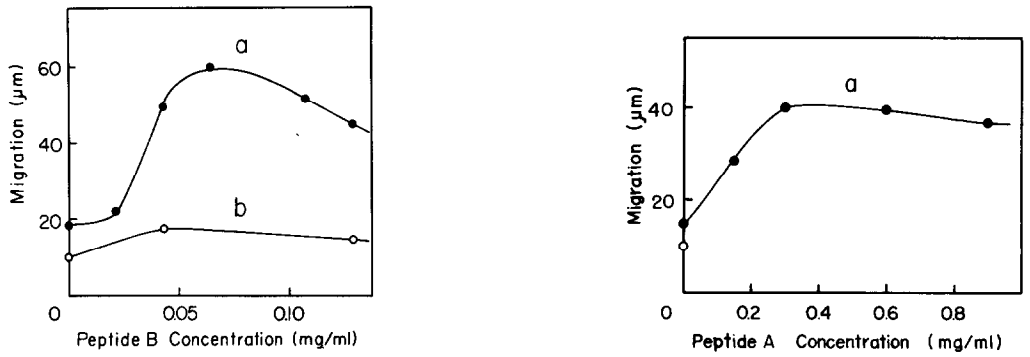


Fig.3. Cooperative participation of two peptides (the peptides A and B) from casein in leukocyte chemotaxis. The left side panel: Curve a; plotting of the chemotactic activity for leukocyte in the presence of 0.3 mg/ml of the peptide A against the concentration of the peptide B. Curve b; in the absence of the peptide A. The right side panel: Curve a; plotting of the chemotactic activity for leukocyte in the presence of 0.064 mg/ml of the peptide B against the concentration of the peptide A. Open circle on the ordinate; chemotactic activity in the absence of both peptides A and B.

of the peptide B against concentration of the peptide A. In the absence of the peptide B, the chemotactic activity did not enhance even in adding a large amount of peptide A. It may be, therefore, concluded that leukocyte chemotaxis was strongly enhanced in the presence of both peptides A and B. Amino acid compositions of the peptides A and B isolated in the present study were determined and shown in table 1. The results were compatible with those of peptides $\beta A_2$ CN1 and $\beta A_2$ CN2 which were named by Ribadeau-Dumas et al. [11], except for a few amino acid residues. The amino acid sequence of the peptide CN1 was from N-terminal Arg through Met 93, which is a strong acidic peptide including phosphate, and that of the other peptide CN2 was from Pro 110 through Met 144, which is rich in hydrophobic amino acid residues [12]. The disagreement for a few amino acid residues may, probably, arise from differences in hydrolytic conditions of

Table 1
Amino acid compositons of peptides A and B

| Amino acid | Peptide A | $\beta A_2$ CN1 (ref. [11]) | Peptide B | $\beta A_2$ CN2 (ref. [11]) |
|---|---|---|---|---|
| Asp | 6.0 | 6 | 1.5 | 2 |
| Thr | 5.2 | 5 | 3.0 | 3 |
| Ser | 6.9 | 8 | 2.3 | 3 |
| Glu | 21.3 | 23 | 5.2 | 5 |
| Pro | 13.8 | 13 | 7.4 | 6 |
| Gly | 2.1 | 2 | 0.1 | – |
| Ala | 1.3 | 1 | 0.1 | – |
| Cys/2 | 0.0 | – | 0.0 | – |
| Val | 5.3 | 7 | 2.4 | 2 |
| Met | 0.0 | 1(HSer) | 0.0 | 1(HSer) |
| Ile | 6.5 | 7 | 0.0 | – |
| Leu | 8.0 | 8 | 6.4 | 7 |
| Tyr | 1.2 | 1 | 0.8 | 1 |
| Phe | 4.0 | 4 | 1.5 | 2 |
| Lys | 4.1 | 4 | 0.9 | 1 |
| His | 1.2 | 1 | 2.2 | 1 |
| Arg | 1.8 | 2 | 0.1 | – |
| Trp | – | – | – | 1 |

peptides between Ribadeau-Dumas' and our experiments, and from genetic variant of β-casein used. For instance, the number of histidine residues of β-casein used in this experiment is 6 instead of 5 in β-casein $A_2$. The experiments are now in progress to test the effect of other peptides except the peptides A and B on leukocyte chemotaxis.

## References

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