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Location of penicilloyl groups on CNBr fragments of the albumin from penicillin-treated patients

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Two fixation sites for penicilloyl groups on human albumin were demonstrated. Using CNBr cleavage the first site was located between methionine 123 and methionine 297 and the second one between methionine 297 and the C-terminal residue. In both cases, penicilloyl groups were unmasked by pronase degradation or disulfide bond reduction.

Albumin; Penicillin; Penicilloyl group; (Human serum)

1. INTRODUCTION

Bisalbuminemia due to the presence of a fast albumin component was observed in patients receiving large amounts of penicillin [1]. This transient bisalbuminemia was shown to be due to the presence of penicilloyl groups on fast albumin [2]. In this work the location of penicilloyl sites within fragments obtained by CNBr cleavage is reported.

2. MATERIALS AND METHODS

Benzylpenicillin sodium and penicilloyl ϵ-aminocaproate (PϵAC) were from Specia.

Crystallized human serum albumin (HSA), 100% pure by electrophoresis, was purchased from Mann. Crystallized ovalbumin (OVA), 100% pure, was obtained from Sigma. After dialysis against water the two proteins were lyophilized.

Penicilloylated ovalbumin (POVA) was prepared by the same procedure as used by Wal et al. [3] for preparing penicilloylated bovine γ-globulin. POVA was partly insoluble and centrifuged before use.

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Serum was collected from a patient receiving intravenously 50×10^6 IU of penicillin G per day for 40 days. Three successive samples were collected at 1 week intervals at the end of the treatment. The samples were mixed and normal and fast albumin were prepared by chromatography on DEAE-Sephadex as described by Lapresle and Wal [2].

CNBr fragments of fast albumin were prepared as specified by Lapresle and Doyen [4]. The reduction of the fragments with β-mercaptoethanol was carried out according to Crestfield et al. [5].

Pronase B grade, the bacterial protease from *Streptomyces griseus*, was obtained from Calbiochem. Degradation of albumin or its fragments with this enzyme was performed as reported by Lapresle and Wal [2].

Alkaline phosphatase (AP) from *Escherichia coli* was obtained from Sigma. Penicilloylated alkaline phosphatase (PAP) was prepared as described by Lapresle and Lafaye [6]. The number of penicilloyl groups per molecule of AP, measured by RIA, was equal to 0.5.

Anti-penicilloyl antibodies were raised in rabbits with bovine γ-globulin coupled to penicillin as described by Wal et al. [3]. Two immunoadsorbents were prepared by reacting 1 g of Sepharose 4B with either 10 mg POVA or 10 mg HSA following the recommendations of the

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manufacturer (Pharmacia). Antibodies were isolated from the serum by fixation on and elution from the immunoadsorbent prepared with POVA. Further purification was achieved by filtering antibody solution through an immunoadsorbent prepared with HSA. The nonadsorbed fraction was collected, dialysed against 0.15 M NaCl and kept frozen.

PAP and anti-penicilloyl antibodies were used for an enzyme-linked immunosorbent assay described by Lapresle and Lafaye [6]. A reference inhibition curve for the reaction between PAP and anti-penicilloyl antibodies was established with PεAC. Penicilloyl HSA conjugates were not used as a reference as done previously [2], since inhibition varies slightly with the amount of penicilloyl groups present on HSA [7].

3. RESULTS AND DISCUSSION

Fast albumin was isolated from the serum of a penicillin treated patient. Its cleavage with CNBr gave rise to three fragments analogous to the fragments obtained from normal albumin. They were named from the N- to the C-terminal ends: B_{1-123}, $C_{124-298}$ and $A_{299-585}$. Furthermore, a portion of fast albumin and of its CNBr fragments were degraded by pronase, whereas another portion of the fragments was subjected to reduction (reduction of HSA was not performed, since it gives rise to insoluble material). All these materials, as well as PεAC, were examined on their capacity to inhibit the fixation of PAP to immobilized anti-penicilloyl antibodies. Fig.1 shows the result of these experiments.

Fast albumin proved to be a better inhibitor than any single fragment and inhibition was markedly increased by its degradation with pronase. The number of penicilloyl groups fixed to HSA was calculated from the amount of pronase-degraded HSA that gave 50% inhibition, using as a reference the amount of PεAC giving the same inhibition. The number of penicilloyl groups per molecule of HSA was found to be 0.9.

No inhibition was observed with fragment B_{1-123}, even after degradation by pronase or reduction of disulfide bonds. Fragment $C_{124-298}$ was inhibitory and this inhibition was greatly increased by degradation with pronase or by reduction. Fragment $A_{299-585}$ was inhibitory and

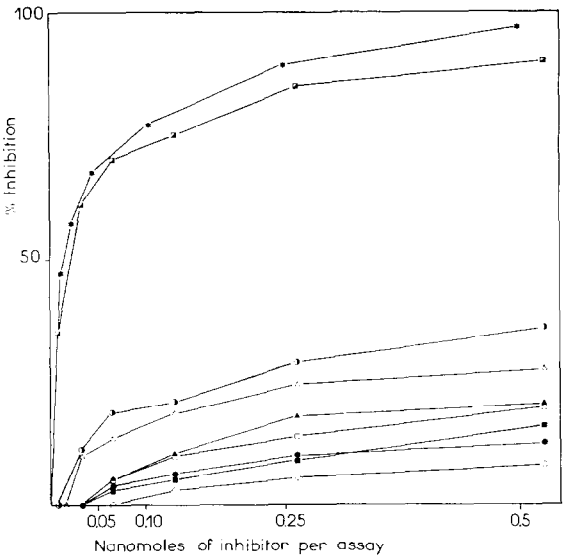

Fig.1. Plates were coated with anti-penicilloyl antibodies and allowed to react with a constant amount of PAP. Inhibition of this reaction by increasing amounts of inhibitors was studied. Inhibitors: fast albumin (◑); fast albumin degraded by pronase (◪); C (○); C degraded by pronase (□); C reduced (△); A (●); A degraded by pronase (■); A reduced (▲); PεAC (★). The quantity of the latter inhibitor is expressed as nanomoles of penicilloyl groups. No inhibition was observed with normal albumin and its CNBr fragments.

inhibition was also increased by degradation with pronase or by reduction but to a lesser extent than that observed with fragment $C_{124-298}$. These experiments show clearly that fixation of penicilloyl groups occurs only to fragments $C_{124-298}$ and $A_{299-585}$.

It was previously shown [2,8] that penicilloyl groups are located on the albumin molecule mainly at sites which were not accessible to antibodies and are unmasked by enzymatic degradation. A similar observation was made with the CNBr fragments. In addition, the reduction was found to be still more effective in unmasking penicilloyl groups than enzymatic degradation, which suggests that the inaccessibility of penicilloyl groups is dependent upon the disulfide structure of the albumin molecule. Unmasking was less complete in the case of fragment $A_{299-585}$ than of fragment $C_{124-298}$ indicating that the penicilloyl site(s) is (are) more readily available on the native molecule in the former case.

The maximum number of penicilloyl groups fixed per molecule of HSA was found to be close to one, in agreement with previous results using radioimmunoassay [2]. Since two sites for penicilloyl groups were demonstrated on HSA in the present paper, penicilloylated HSA should consist of several populations: molecules having non-fixed penicilloyl groups contaminating the fast one, molecules having fixed penicilloyl groups through one or the other of the two sites or through either site. This assumption is consistent with the electrophoretic heterogeneity of fast albumin [2].

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