Monoclonal antibodies block the trypsin cleavage site on human placental alkaline phosphatase

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Received 12 June 1984

Three of eleven monoclonal antibodies (mAbs) to human placental alkaline phosphatase (PLAP) were shown to block cleavage by trypsin at the only proteolytically sensitive site on the native molecule. These results illustrate the potential importance of using mAbs to restrict proteolysis of proteins, in general, and serve as a novel means to identify the relative locations of antigenic determinants.

Monoclonal antibody *Trypsin cleavage* *Restricted proteolysis* *Placental alkaline phosphatase* *Blocking antibody*

1. INTRODUCTION

Since the establishment of the hybridoma technique as in [1], the use of monoclonal antibodies (mAbs) as exquisite reagents in biochemistry has broadened dramatically. Usually the mAb binds to a conformation-dependent determinant at the surface of the antigen making the mAb useful for detection of the native antigen in tissues and biological fluids, and for its isolation by immunoadsorption or immunoprecipitation. The mAbs are also useful in studying structure and function relationships in proteins. Functional properties of the antigen often can be blocked by mAbs allowing the function to be localized to a particular region of the folded polypeptide chain.

The present investigation demonstrates that mAbs can be used to block specific proteolytic cleavage sites of an antigen. These results further add to the usefulness of mAbs as tools in biochemistry.

Human placental alkaline phosphatase (PLAP) in its native form has been shown to be partially cleaved by trypsin, reducing the molecular weight cleaved by trypsin, reducing the molecular mass of the two identical subunits from 67 kDa each to other proteases including chymotrypsin and papain. Several research groups have reported the production of mAbs to PLAP [3–9]. A total of eleven mAbs from two of these groups [4,7,9] are examined here for their ability to block trypsin cleavage of PLAP.

Abbreviations: mAb, monoclonal antibody; PLAP, placental alkaline phosphatase; SDS–PAGE, polyacrylamide gel electrophoresis in sodium dodecyl sulfate

2. MATERIALS AND METHODS

2.1. *Production of mAbs*

Of the 11 mAbs to PLAP, 4 (F11 = IgG_1, D10 = IgG_1, H7 = IgG_{2a}, B10 = IgG_{2a}) were generated against purified PLAP (SS or FS type) [4,7] and 7 (G10 = IgG_{2b}, B2 = IgG_{2b}, H5 = IgG_1, E5 = IgG_{2a}, F6 = IgG_{2a}, C4 = IgG_{2b}, A3 = IgG_1) were generated against HeLa TCRC-1 cells [9], an adenocarcinoma cell line which synthesizes PLAP ectopically [10].

2.2. *PLAP preparation*

The enzyme was purified from an individual SS type human placenta which was homogenized, extracted with butanol, and further processed to homogeneity as reported elsewhere [11]. In some experiments PLAP was radiolabelled with ^{125}I using chloramine T [12].

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00145793/84/$3.00

2.3. *Trypsin digestion*

Trypsin (TPCK-treated) was purchased from Sigma Chemical Co. (St. Louis, MO). PLAP (1 mg/ml) was digested with 2% trypsin in phosphate-buffered saline pH 8.3 at room temperature for 24 h. For several antibody blocking experiments, an equal molar amount of mAb was incubated with PLAP for at least 1 h prior to addition of trypsin. In other experiments, the mAbs were tested for blocking of proteolysis using ^{125}I-labelled PLAP. In these experiments, mAb (10 μg), bovine serum albumin (100 μg), and ^{125}I-PLAP (0.5 μg) were mixed prior to proteolytic digestion.

2.4. *SDS–PAGE*

SDS–PAGE in 10% slab gels was performed as in [13]. Standard markers used included bovine serum albumin (67 kDa), ovalbumin (45 kDa), trypsinogen (24 kDa), and cytochrome *c* (13 kDa). After Coomassie blue staining, the gels were dried and subjected to autoradiography using Kodak XAR-5 film.

3. RESULTS

In its native state, PLAP is cleaved at only one site by trypsin and the major fragment is not further degraded. As seen in fig.1, the molecular mass of the two identical subunits is decreased by only 10 kDa. The smaller fragment is probably further degraded after the initial cleavage since it does not appear in SDS–PAGE either by protein staining or autoradiography when ^{125}I-PLAP is used.

Trypsin also has only a limited effect on the mAbs as seen in fig.2 (lanes 2 and 7). It seems that only the heavy chain is cleaved, probably in the proteolytically-sensitive Fc region which is not involved in antibody binding. Trypsin does not influence the binding between the mAb and PLAP as seen by the ability of E5 to completely protect PLAP from proteolytic attack (lane 3). In contrast, H7 only partially blocks the effect of trypsin (lane 6). Note that E5 and H7 are proteolyzed the same whether or not antigen is present, indicating that the combining sites of the mAbs which would probably protect the antigen are not attacked by trypsin.

A total of eleven mAbs were examined for their

Fig.1. Effect of trypsin on the migration of radiolabelled PLAP in SDS–PAGE. Lane 1, native PLAP; lane 2, trypsin-digested PLAP.

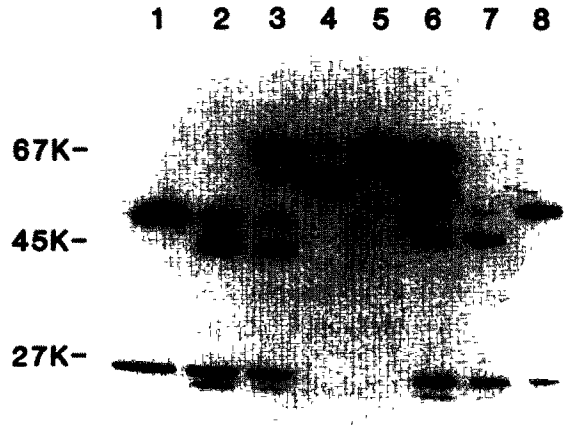

Fig.2. Effects of trypsin on the molecular mass of mAb-free and mAb-bound PLAP shown by SDS–PAGE. Lane 1, E5; lane 2, E5 + trypsin; lane 3, E5–PLAP complex + trypsin; lane 4, PLAP + trypsin; lane 5, PLAP; lane 6, H7–PLAP complex + trypsin; lane 7, H7 + trypsin; lane 8, H7.

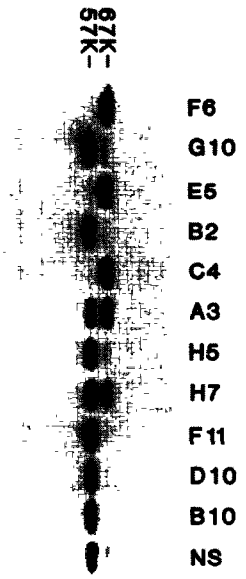

Fig.3. Autoradiography from SDS–PAGE of ^{125}I-PLAP bound to eleven mAbs and then treated with trypsin. NS = nonspecific mouse IgG.

ability to block trypsin cleavage of radiolabelled PLAP as seen in fig.3. The mAbs can be classified into three groups based on their effects on trypsin cleavage: completely blocking (F6, E5 and C4), partially blocking (A3 and H7), and not blocking (G10, B2, H5, F11, D10 and B10).

4. DISCUSSION

Competition experiments assessing the binding of these eleven mAbs to PLAP indicate that F6, E5, and C4, which block trypsin cleavage, probably bind to the same antigenic determinant on the molecule, a site distinct from any which would be occupied by the other eight mAbs [14]. These three mAbs do not bind at the cleavage site but in its vicinity. Indeed, all the mAbs except A3 bind to the 57 kDa fragment produced by trypsin cleavage of PLAP [2], making it unlikely that trypsin cleaves within the antigenic determinant. Thus, the effects of F6, E5, and C4 are probably due to steric hindrance by the antibody polypeptide chains protecting the trypsin cleavage site.

Identification of similar types of blocking mAbs on other proteins may be useful in studies of structure and function relations. By protecting certain proteolytic cleavage sites, one can theoretically engineer a proteolytic digestion to yield more purposefully constructed peptide fragments. Furthermore, the effects of proteolysis on the activation or deactivation of a protein in vivo may be monitored using mAbs which block proteolytic cleavage at a specific site.

ACKNOWLEDGEMENTS

This work was supported by grants CA-21967 and CA-31378 from the National Institutes of Health, US Public Health Service and the Swedish Medical Research Council (4217), the Swedish Cancer Society, and the Medical Faculty, University of Umeå, Sweden. The authors are grateful to Dr William H. Fishman for helpful discussion, Cynthia Page for technical assistance, and Diana Lowe for preparation of the manuscript.

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