DISAPPEARANCE OF ACTIN BINDING PROTEIN FROM HUMAN BLOOD PLATELETS DURING STORAGE

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1. Introduction

During storage, platelets are known to change morphologically from discs to spheres [1,2] and to show a decreased response to aggregating agents [3,4]. These changes on storage are associated with decreased levels of metabolic ATP [5], changes in glycolysis [6] and the loss of certain surface glycoproteins [7].

Here, we present evidence that there is a loss of the actin binding protein of platelets on storage which may also contribute to their reduced response to aggregating agents.

2. Materials and methods

Citrated whole human blood was obtained from the Washington, DC Regional Blood Service – American Red Cross. Within 5 h after donation, platelet-rich plasma (PRP) was prepared by centrifuging the whole blood at 2645 × *g* for 3.5 min. PRP was expressed into a PL-146 blood storage bag made of polyvinyl chloride and equipped with a sampling site coupler to allow easy removal of PRP aliquots under sterile conditions. Each experiment was performed in triplicate using 3 different units of blood.

PRP was stored at 22°C on a rotator moving at 1.2 rev./min. Aliquots (20 ml) were removed under sterile conditions from each of the 3 units every day over 6 days and washed 3 times at 25°C using the method in [8] in a buffer containing 1.1 mM Na_2-EDTA, 96.5 mM NaCl, 85.7 mM glucose and 8.58 mM Tris (pH 7.4). The washed platelets in the above EDTA buffer were adjusted to 10^9 platelets/ml. Phase-contrast microscopic examination showed that > 99.9% of the cells in the final suspending medium were platelets.

The platelets were sonicated using a Sonifier Cell Disrupter (Heat Systems–Ultrasonics, Inc.) and the resulting platelet homogenate was dissolved in an equal volume of a solution containing 2% β-mercaptoethanol, 2% sodium dodecylsulfate and 50% glycerol. Five percent SDS–polyacrylamide gel electrophoresis was run using 100 μg of each sample by the method in [9]. Protein was determined using the Lowry method [10] with bovine serum albumin as standard. Gels were stained with Coomassie brilliant blue (1.25 g) in 8.4% acetic acid, 48.4% methanol and destained in a solution of 5% methanol, 10% acetic acid. The destained gels were scanned for protein at 580 nm on a Gilford spectrophotometer equipped with a gel scanning device.

3. Results

Examination of the SDS–polyacrylamide gel patterns of platelets stored at 22°C as PRP shows the progressive disappearance of two bands of M_r 260 000 and 230 000 and an increase in the intensity and diffuseness of a band with M_r 200 000 (fig.1). Gels similar to those shown were obtained for 10 different units of platelets handled under identical conditions. According to the criteria in [11], the band of M_r 200 000 corresponds to platelet myosin and

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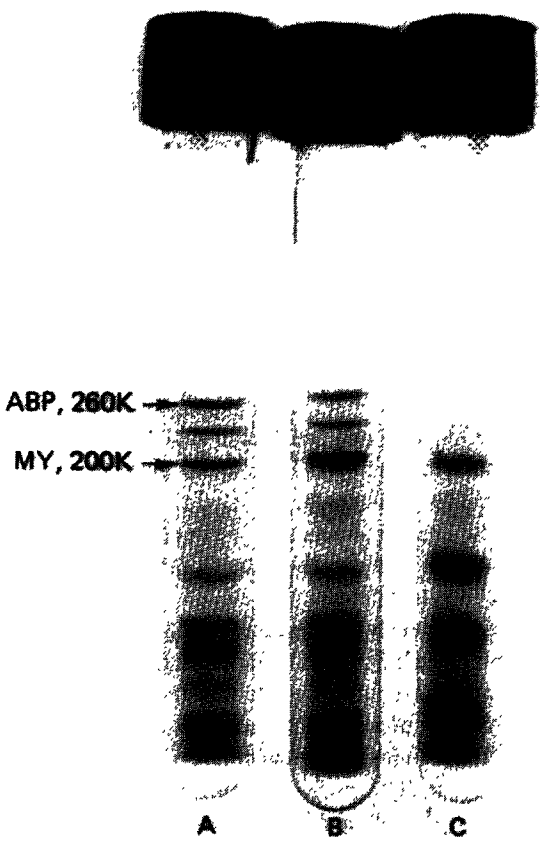

Fig.1. Five percent reduced SDS–polyacrylamide gels run on 100 μg platelet homogenates of platelets which were: (A) fresh, 5 h after venipuncture; (B) 53 h after venipuncture (2 days old) and (C) 125 h after venipuncture (5 days old). ABP, actin binding protein: MY, myosin. Molecular weights were approximated by comparison with high molecular weight standards which were purchased from Biorad. The M_r 240 000 band between ABP and MY is unidentified but also disappears with platelet aging.

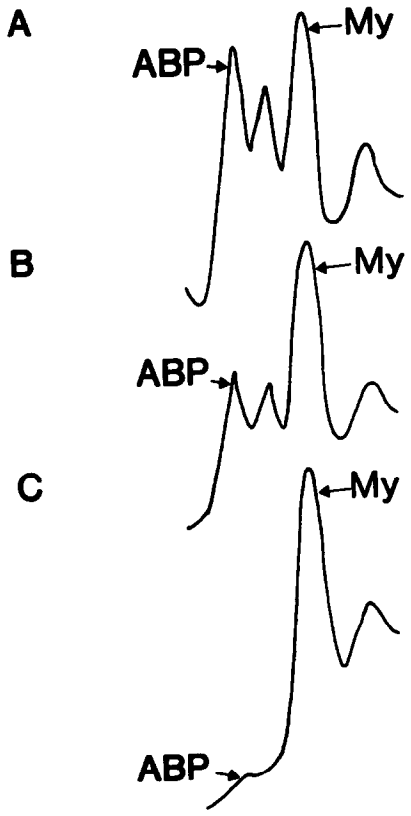

Fig.2. Densitometric scans of the high molecular weight bands shown on the gels in fig.1. Gels were scanned at 580 nm on a Gilford spectrophotometer equipped with a gel scanning device.

that of M_r 260 000 to the actin binding protein; the identity of the band of M_r 230 000 is unknown.

This point is clarified by the gel scans which show the progressive disappearance of the two higher molecular weight bands (fig.2).

The diffuseness in the region of the myosin band (M_r 200 000) was resolved by running the gels at half the protein loading (50 μg). This showed the regular myosin band together with a clearly defined new band of M_r 190 000.

4. Discussion

Actin binding proteins have been found in several systems including chicken gizzard [12], rat kidney fibroblasts [13], guinea pig vas deferens [14] and human blood platelets [11], but the exact role of ABP has not been defined in any of the above systems. Recently, platelet ABP has been quantitatively related to myosin and actin in platelet homogenates which were prepared by different techniques [15]. The present results suggest that there is a decrease in ABP during platelet storage together with the appearance of another band (M_r 190 000) which may arise from it or from the other component (M_r 230 000) which decreases during storage. A Ca^{2+}-

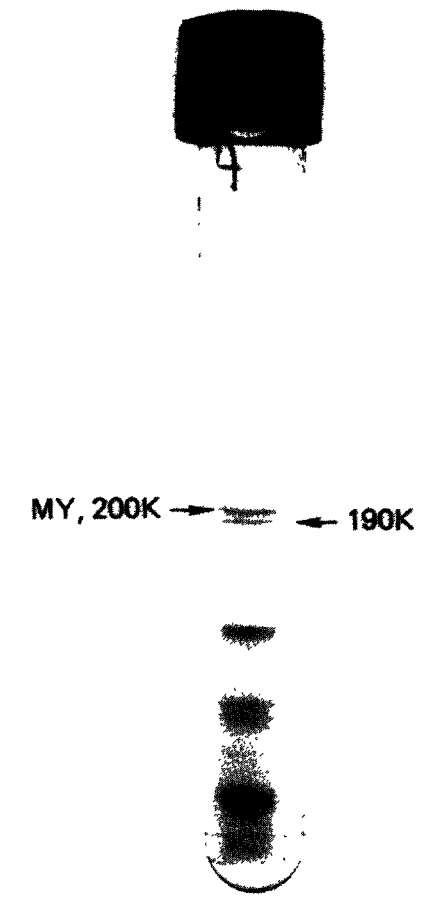

Fig.3. Five percent reduced SDS–polyacrylamide gel run on 50 μg platelet homogenate of platelets which were 5 days old. The M_r 190 000 band, which is not noticed on gels of fresh platelets, is now visible.

dependent protease which has recently been detected in platelets [16] can cleave ABP [17]. Thus, the observed decrease in ABP during platelet storage may reflect the activity of this enzyme.

One of the functions of ABP in platelets appears to be to effect the side-by-side arrangement of fibrous actin in an array similar to the microtubular assemblies which are seen to exist in fresh disc-shaped, unstimulated platelets [18]. Thus, the loss of ABP during storage could determine the loss of reactivity to aggregating agents. In this connection it may be noted that platelets from patients with Glanzmann's thrombasthenia, which fail to respond to aggregating agents [19,20], have recently been shown to lack ABP [21]. Furthermore, ABP has an inhibitory effect on the actin activation of heavy meromyosin ATPase [22]. Thus, degradation of ABP could result in a stimulation of ATPase activity and the loss of ATP, as observed during platelet storage [5].

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