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Pathophysiological significance of protein hydrophobic interactions: An emerging hypothesis

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ABSTRACT

Fibrinogen is a unique protein that is converted into an insoluble fibrin in a single enzymatic event, which is a characteristic feature of fibrinogen due to its susceptibility to fibrinolytic degradation and dissolution. Although thrombosis is a result of activated blood coagulation, no explanation is being offered for the persistent presence of fibrin deposits in the affected organs. A classic example is stroke, in which the thrombolytic therapy is effective only during the first 3–4 h after the onset of thrombosis. This phenomenon can now be explained in terms of the modification of fibrinogen structure induced by hydroxyl radicals generated during the period of ischemia caused, in turn, by the blocking of the blood flow within the obstructed vessels. Fibrinogen modification involves intra-to intermolecular disulfide rearrangement induced by the reductive power of hydroxyl radicals that result in the exposition of buried hydrophobic epitopes. Such epitopes react readily with each other forming linkages stronger than the peptide covalent bonds, thus rendering them resistant to the proteolytic degradation. Also, limited reduction of human serum albumin (HSA) generates hydrophobic polymers that form huge insoluble complexes with fibrinogen. Consequently, such insoluble copolymers can be deposited within the circulation of various organs leading to their dysfunction. In conclusion, the study of protein hydrophobic interactions induced by a variety of nutritional and/or environmental factors can provide a rational explanation for a number of pathologic conditions including cardiovascular, neurologic, and other degenerative diseases including cancer.

Introduction

Hydrophobicity is a physical property of a molecule that is repelled from a mass of water and is responsible for the formation of intermolecular aggregates (micelles). Hydrophobicity of a protein is determined by the proportion of nonpolar amino acids (with aliphatic or aromatic side chains) with respect to those with polar residues [1]. Hence, the most hydrophobic proteins are those containing high content of isoleucine and valine, and those containing negatively charged amino acids are more hydrophilic. Hydrophobic forces in native proteins are involved in maintaining a proper three-dimensional structure of their polypeptide chains linked by the disulfide bonds. But, any disruption of such bonds is associated with the exposure of *intra*molecular hydrophobic groups and their subsequent *inter*molecular interactions. Consequently, huge hydrophobic aggregates are being formed that are expelled from an aqueous medium usually with the formation of insoluble polymers or gels.

The incubation of a perfectly soluble human serum albumin (HSA) with strong reducing agents, such as dithiothreitol (DTT) and/or homocysteine (HC), resulted in the formation of an insoluble polymer (gel) (Fig. 1). Although the formation of such a polymer can be prevented by the redox active sodium selenite, once the gel is formed it cannot be disrupted by breaking the disulfide bonds of the polypeptide chains of HSA. Moreover, insoluble polymers are resistant to the action of proteolytic enzymes, e.g., chymotrypsin, similar to that induced in fibrinogen with redox iron [2]. The only bonds that would explain this phenomenon are those resulting from the hydrophobic interactions known to be the strongest forces found in the living organisms [3–5]. It is of extreme importance to note that a physiological reducing agent, homocysteine (HC), similar to DTT, has been found to be significantly increased in the plasma of patients with the degenerative diseases [6,7] This fact can explain the formation of insoluble protein aggregates in human body known as *amyloids* [8–10] and biofilms in bacterial colonies [11]. Homocysteine inhibits the fibrin clot lysis, most likely by altering its internal structure [12] or by the formation of a complex with lipoprotein [13,14].

Amyloidosis refers to several disorders caused by misfolding of normal cellular proteins into highly β-sheet-rich insoluble aggregates

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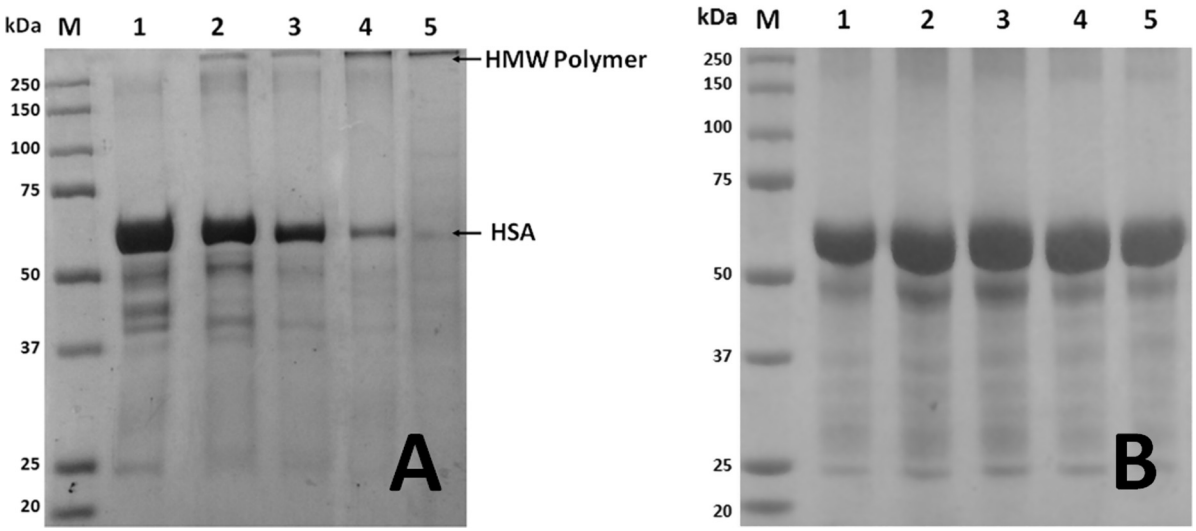

Fig. 1. Electropherogram of human serum albumin 5% treated with 100 m homocysteine after different times of incubation at 37 °C (M: marker, 1: 1 h, 2: 2 h, 3: 3 h, 4: 4 h, 5: 5 h). Decreased intensity of human serum albumin (HSA) – band, and the appearance of high molecular weight (HMW) polymer at the top at gel are visible (A). Note that the polymer ins not reducible under the condition of SDS-PAGE. Incubation of the same system in the presence of 100 m sodium selenite prevented the formation of the HMW polymer (B).

and their subsequent deposition in various parts of the body [15,16]. More than 20 unrelated proteins can form amyloid fibrils in *vivo*, which are related to various diseases, such as Alzheimer's disease, prion disease, and systematic amyloidosis. A transition of amyloidogenic protein by alternative folding pathway under certain conditions leads to the formation of protease-resistant amyloid fibrils, having predominantly cross β structure. Insulin is particularly prone to the formation of inactive amyloid fibrils, which may explain the relationship between free radical stress and diabetes mellitus [17,18]. Insulin and other protein amyloids can be dissociated by the non-proteolytic enzymes, such as serrapeptase, lumbrokinase, and nattokinase, making them potential drug candidates for the treatment of a number of amyloid-related diseases [19,20]. Implications of protein hydrophobic interactions in numerous degenerative diseases are discussed below.

Hypothesis

Hydrophobic polymers can coat cell membranes and make them resistant to the recognition and destruction by the innate immune system. This process can be inhibited by the treatment of specific pathologies with the redox-active selenium, as well as by the amphiphilic natural polyphenols. But, once the protective hydrophobic coat is formed it cannot be degraded by serine proteases, but it can be achieved by the enzyme *Nattokinase* present in the fermented soy products.

Cardiovascular disease

Accumulating evidences from last several decades points out to the important role of hemostatic abnormalities in the pathogenesis of atherosclerosis and cardiovascular disease (CVD). For example, cigarette smoking is known to contribute to atherosclerosis by altering the fibrin clot dynamics and architecture, which may also be caused by hydroxyl radicals generated under hypoxic conditions. The higher the fibrinogen concentration the greater is the chance of thrombus formation, therefore everything should be performed to lower the blood concentration. Although it is a desirable goal, the critical factor in thrombosis is not the amount of thrombin-clottable protein, but it is how long the thrombus persists in the circulation. Inhibition of fibrin degradation in atherosclerotic lesions may be caused by the presence of free radical-modified human serum albumin [21,22] that when hydrophobically bound to fibrin makes it resistant to fibrinolysis. This notion is supported by the findings of Undas et al. [23], who have demonstrated the reduced fibrin clot permeability and its resistance to fibrinolytic degradation in association with the generation of free radicals [24,25].

In view of the powerful fibrinolytic potential of human blood, the mere activation of blood coagulation does not offer a satisfactory explanation for thrombosis. The presence of fibrin-like material in atherosclerotic plaques was first observed by Rokitansky over 150 years ago and a century later confirmed by Duguid [26,27]. The existence of fibrin(ogen)-related antigens in the insoluble deposits of atherosclerotic intima [21] indicates that these deposits are resistant to fibrinolysis, thus suggesting the presence of hydrophobic protein complexes [28]. Further evidence for role of hydroxyl radicals in the pathogenesis of atherosclerosis and CVD is indirectly obtained in an experimental study, in which a potent scavenger, edaravone, was shown to significantly reduce the size of myocardial infarct in rabbits [29]. In addition, a higher prevalence of plaque iron may contribute to the increased incidence of atherosclerosis by participating in the hydroxyl radical production [30]. Therefore, the preferred strategy for the prevention of atherosclerosis is by the administration of iron chelating agents and direct hydroxyl radical scavengers in combination with the hydrophilic substances (e.g., polyphenols) that can counterbalance the consequences of protein hydrophobic interactions. Cardioprotective effect of highly hydrophilic docosahexaenoic acid [31] can also be explained by its hydroxyl radical scavenging properties [32,33].

Cancer

Neoplasia and uncontrolled cell growth is another example of a pathologic condition in which insoluble fibrin(ogen) deposits have been implicated. An increased tendency to thrombosis in cancer was observed over one hundred years ago, but no complete explanation of this phenomenon is yet available. Thus, anticoagulation with warfarin is practically ineffective in patients with breast cancer [34]. Even more important was the observation of the deposition in cancer tissue of fibrin-like material without thrombin generation [32–35]. Therefore, this obvious paradox can only be explained in terms of fibrin(ogen)