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Ischemia-modified albumin: Crosstalk between fatty acid and cobalt binding

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ABSTRACT

Myocardial ischemia is difficult to diagnose effectively with still few well-defined biochemical markers for identification in advance, or in the absence of myocardial necrosis. "Ischemia-modified albumin" (IMA), a form of albumin displaying reduced cobalt-binding affinity, is significantly elevated in ischemic patients, and the albumin cobalt-binding (ACB) assay can measure its level indirectly. Elucidating the molecular mechanism underlying the identity of IMA and the ACB assay hinges on understanding metal-binding properties of albumin. Albumin binds most metal ions and harbours four primary metal binding sites: site A, site B, the N-terminal site (NTS), and the free thiol at Cys34. Previous efforts to clarify the identity of IMA and the causes for its reduced cobalt-binding capacity were focused on the NTS site, but the degree of N-terminal modification could not be correlated to the presence of ischemia. More recent work suggested that Co^{2+} ions as used in the ACB assay bind preferentially to site B, then to site A, and finally to the NTS. This insight paved the way for a new consistent molecular basis of the ACB assay: albumin is also the main plasma carrier for free fatty acids (FFAs), and binding of a fatty acid to the high-affinity site FA2 results in conformational changes in albumin which prevent metal binding at site A and partially at site B. Thus, this review advances the hypothesis that high IMA levels in myocardial ischemia and many other conditions originate from high plasma FFA levels hampering the binding of Co^{2+} to sites A and/or B. This is supported by biophysical studies and the co-association of a range of pathological conditions with positive ACB assays and high plasma FFA levels.

1. Introduction

Myocardial ischemia occurs due to restricted blood supply to the muscular tissue of the heart (myocardium) resulting in insufficient oxygen supply. The main cause of this can be the partial or complete blockage of a coronary artery, and a critical depletion of myocardial oxygen leads to cell death, or infarction. Diagnosis of myocardial ischemia typically includes exercise-electrocardiography stress tests, coronary angiography, and imaging stress-echo tests [1]. While a plethora of cardiac biomarkers have been described for detecting the development of other acute coronary syndromes (ACS) [2,3], there are still few well-defined biochemical markers for identification of myocardial ischemia in advance, or in the absence of myocardial necrosis. One of these biomarkers is based on albumin, the most abundant protein in blood plasma. So-called "ischemia-modified albumin" (IMA) is found to be significantly elevated in ischemic patients [2,4–7], and serves as a biomarker for early detection of myocardial ischemia before the onset of irreversible cardiac injury [6]. IMA is solely characterised by its reduced cobalt-binding affinity, which can be measured indirectly by the Food and Drug Administration-approved albumin cobalt-binding (ACB) assay [8,9].

In the commercially available ACB test, cobalt(II) chloride (approximately 1.5 mol equivalents per albumin molecule) is added to a serum sample, to allow albumin-cobalt binding. Dithiothreitol (DTT), a metal chelator that forms a coloured complex with Co^{2+}, is then added. The resulting ill-defined brown DTT-Co^{2+} product is measured by absorption spectrophotometry at 470 nm and compared to a serum-cobalt blank without DTT present. The reduced cobalt-binding capacity of IMA

Abbreviations: ACB, albumin cobalt-binding; ACS, acute coronary syndromes; ATCUN, amino terminal Cu(II) and Ni(II) binding motif; DTT, dithiothreitol; EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure spectroscopy; FFAs, free fatty acids; HRG, histidine-rich glycoprotein; IMA, ischemia-modified albumin; ITC, isothermal titration calorimetry; NMR, nuclear magnetic resonance; NTS, N-terminal binding site on albumin

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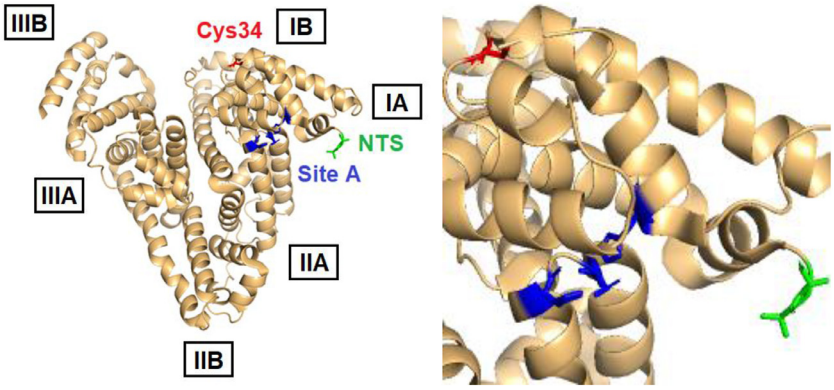

Fig. 1. Location of the three metal binding sites that have been successfully identified on human serum albumin,PDB: 5IJF [60]. Site A, the multi-metal binding site (MBS) (blue); NTS/ATCUN motif (green); Cys34 (red). The precise location of site B is not yet known. The boxed labels indicate the six sub-domains of albumin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leaves more unbound Co^{2+} to complex with DTT, resulting in higher absorbance readings [10]. The ACB test has an excellent negative predictive value, *i.e.* low IMA readings correspond well to the absence of myocardial ischemia. However, a severe shortcoming is the high incidence of false positives, *i.e.* high readings in the absence of ischemia.

After its first description [8], the molecular identity of IMA remained elusive. Based on the general assumption that Co^{2+} would preferentially bind to an N-terminal site [11–13], efforts to elucidate the molecular causes of reduced cobalt binding concentrated on this site. It was hypothesized that ischemia causes the N-terminal end of the albumin protein to undergo structural modifications, hence that IMA corresponded to N-terminally modified albumin [13]. The structural modifications proposed and investigated included cleavage of the first two residues and oxidation [11], which were suggested to result from free radical damage, exposure to free iron and copper, or disruption of ion pumps [8,14].

However, in-depth studies could not reveal a correlation between N-terminal modifications and ACB readings [13,15]; more recently, no correlation was found between the ACB assay and an enzyme-linked immunosorbent assay that specifically detects N-terminal modification of albumin in patients with either acute coronary syndrome or non-ischemic chest pain [16]. Similarly, patients suffering from acute-on-chronic liver failure have significantly elevated ACB assay readings, but the same proportion of N-terminally modified albumin as healthy individuals [17,18]. In the light of such findings, low plasma pH as a result of acidosis, and altered plasma cysteine/cystine ratio as a consequence of hypoxia or oxidative stress have also been suspected as molecular causes of reduced cobalt binding [19]. The need to consider the contribution of other plasma components to the Co-DTT complex formation was also highlighted [19]. Indeed, a positive correlation has been identified between the highly elevated serum levels of free fatty acids (FFAs) in patients with acute ischemic myocardia and high levels of IMA [20]. Following our discovery of FFA-mediated inhibition of zinc binding to albumin [21–24], we have demonstrated that the conformational changes that FFA-binding to albumin elicits in the protein is sufficient to cause reduced cobalt binding capacity [22,25]. This review will present essential background information on metal ion-albumin interactions and discuss the molecular basis of FFA-mediated inhibition of metal (in particular Co^{2+}) binding. It will also provide a clinical perspective to highlight how conclusions from biochemical/bioinorganic investigations are reflected in patient data.

2. Albumin – a carrier of essential and xenobiotic metal ions in plasma

Albumin is a ~66 kDa protein containing 585 amino acids, contributing to around 50% of the total protein concentration in blood plasma, and up to 75% of the colloidal activity [26]. Albumin comprises three homologous but structurally distinct domains, each divided into two sub-domains [27]. One of its key roles in the body is to transport a variety of small molecules, including cholesterol [28], fatty acids [29], and pharmaceutical drugs [30]. Importantly, albumin also serves as an important carrier of inorganic ions, including those required for regular physiological function (Ca^{2+}, Cu^{2+} Zn^{2+}) [31], toxic metal ions (Cd^{2+} and Ni^{2+}) [32,33], as well as metal-based therapeutics (Au^{+} and Pt^{2+}) [34,35]. Before considering cobalt binding in depth, we will briefly summarise the interactions of albumin with other d-block metal ions, with the exception of Cr^{3+}, Fe^{3+}, and Mn^{2+}, which are preferentially transported by transferrin, another important metal ion transporter in blood plasma. Whilst Fe^{3+} can, in principle, also bind to albumin, this only occurs in cases of severe iron overload [34].

2.1. Metal binding sites in serum albumins

Though originally albumin was thought to transport ions in a non-specific 'sponge-like' manner [30], four partially selective metal binding sites have been identified, namely the N-terminal site (NTS), sites A and B, and Cys34 (Fig. 1) [34]. Metal binding to such sites can be studied using a variety of techniques. Stability constants for the binding of d-block metals, including Zn^{2+}, Cu^{2+}, Ni^{2+} and Cd^{2+}, were originally derived from equilibrium dialysis experiments [36-39]; more recently, isothermal titration calorimetry (ITC) has provided valuable thermodynamic data for metal ion binding [40]. Nevertheless, both of these techniques only provide global binding constants [34] and need to be complemented by techniques that address structural features. For true transition metal ions such as Cu^{2+} and Co^{2+}, electronic spectroscopic methods such as circular dichroism allow metal binding to albumin to be studied via transfer of chirality from metal-binding amino acid residues to the d-d/charge-transfer bands of complexed metal ions, providing insight into the geometry of metal-protein interactions [41,42]. The same ions have unpaired electrons, and can also be investigated using electron paramagnetic resonance (EPR) spectroscopy, which provides insight into the chemical environment surrounding the metal ion [43,44]. To obtain structural information on the binding of diamagnetic d^{10} ions, such as Zn^{2+} and Cd^{2+}, that are largely silent in the aforementioned spectroscopies, nuclear magnetic resonance (NMR) methods have been employed, making use of either partially-characterised ^{1}H-resonances of metal-binding residues, or NMR-active nuclei such as the ^{111}Cd or ^{113}Cd isotopes of cadmium [39,45–47]. Further information on the coordination mode, geometry and identification of likely donor ligands has been gained using extended X-ray absorption fine structure spectroscopy (EXAFS) [47]. In addition, mass spectrometry has been used as a tool to detect crosslinking of His67 and His247 by platinum in site A [48].

2.1.1. The N-terminal binding site (NTS)

One of the first metal binding sites to be identified on albumin was the N-terminal binding site (NTS), which arises from the first triplet amino acid motif of human albumin: Asp1–Ala2–His3 (Figs. 1 and 2) [49]. It involves the N-terminal amino group, the N(delta) of His3, and