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Review article

Targeting HbS Polymerization

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ABSTRACT

The mutation of β6 from glu to val in hemoglobin is responsible for the polymer formation that leads to vaso-occlusion, and a range of severe consequences in sickle cell disease. The treatment of the disease can be addressed in many ways, but the prevention of polymer formation is one of the most fundamental approaches one can take. Such prevention includes affecting the polymer structure, or dilution of the fraction of polymerizable hemoglobin. The latter approach includes (1) induction of HbF, which does not itself, nor in hybrid form, join sickle polymers, or (2) restricting the allosteric change in hemoglobin that occurs in oxygen delivery, and which is required for polymer formation. These approaches will be critically reviewed, as well as the most recent developments that show the benefits of simply swelling the volume of the red cell.

Introduction

The mutation at the sixth position of the hemoglobin β chain that replaces a glu with a val (negative charge to hydrophobic) results in a molecule that still cooperatively binds oxygen, but can now form rigid, 14-stranded polymers in the fully deoxygenated state. This sickle-cell mutation wreaks havoc on the lives of millions throughout the world with recurrent pain, chronic organ damage, and premature death. Preventing the formation of such polymers unequivocally prevents the problems of the disease, and thus it is helpful to summarize the main principles on which polymer-preventing therapies are based as well as their current state of implementation.

There are 3 Prerequisites for Occlusion

The problems created by polymerization are a consequence of the ability of rigid cells to obstruct circulation, and deprive tissues of oxygen. This requires that sufficient polymers form, and that they do so rapidly enough to jam a red cell behind an adherent erythrocyte or leukocyte attached to the postcapillary venule wall. We consider these requirements in turn.

There Must be Enough HbS Polymerized

The propensity for different forms of HbS (liganded, modified, hybridized, etc.) to polymerize is measured by a determination of their solubility, c_s, also often called c_{SAT}, the concentration of monomers that must remain in solution. Given c_s one can determine how much polymer will form (though as we shall see, the rate of its formation is critical). The amount of hemoglobin that polymerizes is dictated by difference between the initial concentration and the solubility [1]. That difference is the thermodynamic maximum. It has been found that formation of a dense mass of polymers obstructs their growing ends and traps the net polymer mass at about only two-third of the thermodynamic maximum until either, at longer times, random motion eventually allows the polymers to elongate, or until an external force such as centrifugation fractures the polymers and releases the obstructions [2].

The resulting rigidity of the cells depends on the net polymerized mass. It has been found that rigidity (Young's modulus) rises as the square of the polymer mass [3]. Small amounts of polymer are thus tolerable without causing obstruction.

Polymerization Must Occur Fast Enough to Cause Obstruction

The molecular mechanism by which the formation occurs is understood in great detail [4,5]. The steps toward forming polymers are not all favorable. Energetically this means the initial steps are not downhill, implying the presence of a barrier that needs to be surmounted before polymer growth can be sustained. The first polymers in a solution form by random coalescence of enough molecules (the nucleus) that lets the subsequent additions to that nucleus all be favorable. That step, called homogeneous nucleation because it happens in homogeneous solutions, is complemented by a process called heterogeneous nucleation whereby existing polymers catalyze the nucleation of new polymers on their surface. The feature most critical for understanding the pathophysiology is the time during which the polymers formed are few

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enough to give the illusion that nothing is happening, and that the reaction has been "delayed" [6].

This "delay time," in accord with the above observation of the importance of polymer mass, grants an effective grace period during which a cell can still escape the microcirculation without incident even though it has begun the downhill slide that will lead to its rigidification.

Polymerization is Strongly Dependent on the Concentration of deoxyHbS

One of the most striking features of earliest work on the kinetics of HbS polymerization was the extraordinary dependence of delay time on initial concentration. For example, a mere 2% dilution would suffice to double the delay time [7]! Once techniques had been developed that allowed subsecond measurements, it became clear that the high concentration dependence tapered off somewhat, to require instead a 4% dilution for the aforesaid delay time doubling [8]. Those measurements also revealed that the nuclei, which control the rate of polymer formation, are actually in the range of pentamers under physiological conditions, rather than the much larger entities previously thought. It should be noted, though, that these numerical values pertain to fully deoxygenated solutions, and the presence of oxygen or other ligands also lower the rates and increase nucleus size [9]. Replacing deoxyHbS by oxyHbS, however, is not the same as simple dilution because of molecular crowding.

Molecular Crowding Profoundly Affects the Rates

The fact that a substantial fraction of space is taken up by Hb molecules means that the surfaces at which the interactions will occur are significantly closer to one another than the separation of the molecular centers. This is known as nonideality or molecular crowding. Where chemical reactions would employ concentration for the calculation of equilibrium, here one must use activity, which is defined as the product of an activity coefficient γ times the concentration. For monomers, the activity coefficient follows a simple formula, viz.

$$\ln \gamma = 8\phi/(1-\phi)^2$$

where γ is the volume fraction, that is, $\phi = vc$ where c is concentration and v is the specific volume of a hemoglobin molecule [10]. But more than simply causing one to recalibrate concentrations, crowding is important because species that do not polymerize nonetheless can have a significant effect on the rates of reactions because of the space they occupy. Oxygenated HbS molecules, or HbF molecules, will thus play a significant role as enhancers by simply taking up space. Thus, for example, in the course of a cell's deoxygenation process, the activity coefficients remain constant, since they depend on the total concentration of volume occupiers, not their identities.

To illustrate the magnitude of these effects, consider the rate at which homogeneous nuclei form [11]. At physiological concentrations, if one were to decrease the Hb concentration by 30%, the rate would go down by approximately a factor of 10^8. But if one simply replaces 30% of the Hb with molecules of the same size that are incapable of polymer formation, the decrement is only a factor of 10. Molecular crowding is responsible for the difference.

Delay Time Behavior is Conveniently Summarized by a Supersaturation Rule

The use of knowing the delay times has led to an empirical scaling law, by which one can relate delay time to the supersaturation of the solution [12]. Supersaturation is the ratio of the monomer activity, γ divided by the solubility times its own activity coefficient, that is, $\gamma_s c_s$. This supersaturation relationship could be especially useful, for example, if one wishes to extrapolate high phosphate data to delay times under low phosphate conditions. Once the solubilities at low and high phosphate are known, the delay times will follow. Moreover, even though changing the delay time is of paramount importance, the discussions that follow below will mainly focus on how one effectively changes the solubility, due to the interrelationship of the delay time and solubility.

This curve relates the delay time, a kinetic parameter, to a thermodynamic driving term, which is often easier to deduce in a therapeutic design. Physiologically, cells take on the order of a second to transit the microcirculation [13-15]. On the other hand, due to finite oxygen delivery rates, it is hard for a cell to sickle must faster than half a second [16].

Quantitatively, this delay time supersaturation curve is quite striking. Around physiological concentrations and saturation (so that delay times are in the 1 second range), a change in concentration of the solution (or cell) of 10% changes delay times by a factor of around 16. A change in solubility of 10% changes delay times by about a factor of 5. This is a particularly encouraging feature, since one can imagine that changes in solubility that might appear rather modest could actually be clinically significant.

An Adhesive Cell Must Reduce the Postcapillary Lumen

When a cell polymerizes within a capillary, the forces that might act to keep it stuck there appear to be insufficient to prevent it being forced out by typical arterio-venous pressure differences according to measurements made on cells immobilized in microfluidic channels, in agreement with theoretical expectations [17]. However, the rigidified cell cannot deform further: it will take the shape of the capillary in which it polymerized and retain it. Postcapillary venules have been observed to be the site of adherent erythrocytes of leukocytes [18-20]. This would not represent a problem for a deformable cell, which could easily slip past such a partial obstruction, but a rigidified cell, faced with the need to deform from a round cross section to a flatter and wider aspect to navigate the changed cross section, can no longer do so. Although they will not be considered here, strategies to eliminate these adherent events may also be productive therapeutic directions.

Much About the Polymer Structure is Established, But Important Details Remain Unknown

Knowledge of the polymer structure might provide possible therapeutic targets for drugs that would directly disrupt polymer formation. Careful electron microscopy has established that the polymers typically possess 14 strands, formed in a twisted, rope-like structure [21]. Atomic level information is only available at present from model building studies [22-24], based on the electron microscope reconstructions combined with hypothesized helical distortion of the observed linear structure of the crystals formed from deoxyHbS [25,26]. The crystals entail a linear double strand structure, with one of the 2 mutant β6 val molecules snugly docked in a hydrophboic pocket receptor region on other β chains. This is known as the lateral contact, and forms a zig-zag pattern along the double strand. The hydrophobic contact is augmented by a salt bridge formed between His α 50 and Asp β 79 in the lateral region as well [26]. (This makes solubility pH dependent.) There are also axial contacts made between the molecules that employ a diffuse and irregular network of hydrophobic contacts between