Physiology

Haemoglobin's chaperone

Lucio Luzzatto and Rosario Notaro

Molecular chaperones come in different forms, but all have a similar task: to keep other proteins in shape. A newly identified chaperone seems to be specific to haemoglobin, preventing precipitation.

The rate of production of consumer goods is controlled at the assembly line, but it must be also adjusted according to demand. In biological terms, the synthesis of haemoglobin is an extreme example of what happens when market demands are high. Haemoglobin is the protein that makes red blood cells red. It is responsible for delivering oxygen to all tissues and organs in the body, and to do so optimally it accumulates in red blood cells to an incredible 340 grams per litre[1]. In adults, functional adult haemoglobin (HbA) consists of two α- and two β-globin chains, each containing haem — a non-protein group that carries oxygen[2]. The two chains cooperate to ensure that HbA can bind and release oxygen in an efficient and well-controlled way.

The control of globin gene expression is complex[3,4], and the need for two different globin chains makes the assembly line even more complicated, especially because the two genes are on different human chromosomes[5,6]. The end result is a high rate of haemoglobin production, with absolute tissue specificity. A long-standing question[7] is how red blood cells ensure that both globin genes are top performers and yet provide their protein products in perfect stoichiometry. On page 758 of this issue, Kihm and colleagues[8] provide at least part of the answer.

Among several differences between the α- and β-globin chains, two are especially important. At the genomic level, there are two α-globin genes to every β-globin gene. At the protein level, the β-chain can associate on its own with haem and form a tetramer, called HbH. This tetramer is functionally useless because, although it does bind oxygen, it cannot easily release it. By contrast, haem-bound α-chains on their own tend to form precipitates, called α-inclusion bodies, that damage red blood cells[9].

We can now perceive how stoichiometry might work. If red blood cells produced β-chains in excess, there would be wasteful synthesis of HbH. So one can imagine that it would be advantageous for α-chains to be produced in slight excess, and there is experimental evidence that this does indeed happen. Thus, α-chains will combine with every available β-chain to form functional HbA. But this arrangement will work only if the intrinsic instability of the excess α-chains does not cause harm to the cell or cell membranes. Kihm *et al.*[8] now show that a

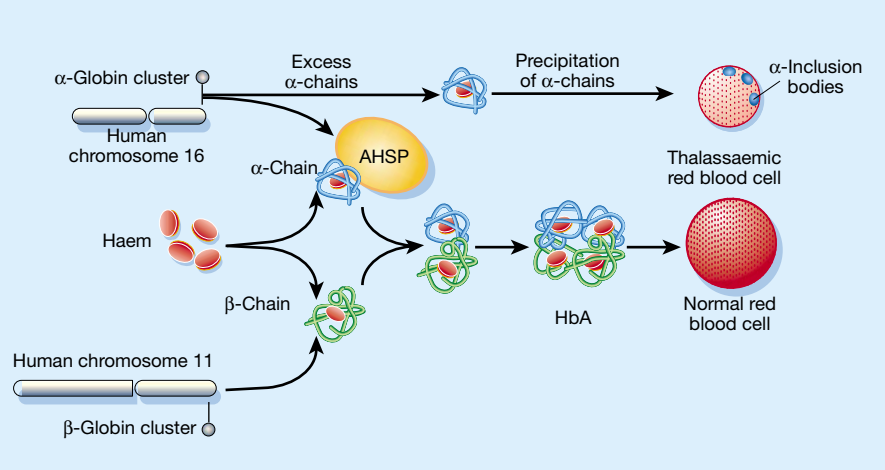

Figure 1 **Balancing the components of haemoglobin. Left, the α-globin and β-globin chains are encoded by genes on different chromosomes and so their expression (which is specific to red blood cells) is controlled independently. Slightly more α-chains than β-chains are produced (not shown). Having incorporated the oxygen-carrying haem group, the α- and β-chains cooperate to form first dimers and then tetramers (lower pathway). Kihm *et al.*[8] find that AHSP binds specifically to α-haemoglobin, and suggest that it might thereby serve a dual purpose — to stabilize these chains and to help in delivering newly formed α-chains to β-chains. Top pathway, in β-thalassaemia fewer β-chains are produced and the excess in α-chains is too great for AHSP to handle; some α-chains precipitate, damaging the cell. The involvement of AHSP in the production of haemoglobin is corroborated by the finding[8] that red blood cells from AHSP-deficient mice have features reminiscent of thalassaemia.**

100 YEARS AGO

We should like to draw your attention to the following spectacle which some of us witnessed on the sea-shore at Blundellsands on Thursday evening, June 5, at about eight o'clock. The evening was dull and grey, a strong north-westerly wind was blowing in from the sea and the tide was flowing in. In the distance we first saw smoke with frequent jets of fire bursting forth from the mud of a shallow channel. Drawing near, we perceived a strong sulphurous odour, and saw little flames of fire and heard a hissing sound as though a large quantity of phosphorus was being ignited. It was impossible to detect anything which caused the fire... The area over which the tiny flames kept bursting forth was about 40 yards. A gentleman present stirred up the mud with his walking-stick, and immediately large yellow flames nearly 2 feet in length and breadth burst forth. The phenomenon lasted some time, until the tide covered the part and quenched the fire.

From *Nature* 12 June 1902.

50 YEARS AGO

In Sweden, as in Britain, observations have been made of the opening of milk bottles by blue tits and great tits... Although no experimental analysis of the behaviour involved in the opening of milk bottles has yet been made, further observations in the field enable the discussion to be carried further. Previously the processes were considered in two parts — the 'recognition' of the milk bottle as a potential supply of food and the technique of opening the bottle. The actual opening of the bottle probably depends on innate motor patterns, and the study of tits in the field has confirmed this view... It has been suggested that the initial 'discovery' of the bottle as a source of food may be a logical consequence of the feeding habits of tits... In fact, it seems that, when tits are looking for food, objects with certain very general characteristics may be sufficient to release a more limited type of searching behaviour (such as flying to the food). By a succession of similar steps (hammering, etc.), evoked by successively more specific stimuli, a reward may eventually be obtained. It so happens that milk bottles have sufficient characteristics in common with the natural foods for tits occasionally to 'discover' them in the course of their normal searching.

From *Nature* 14 June 1952.

previously discovered protein, which they call α-haemoglobin-stabilizing protein (AHSP), seems to prevent just this problem (Fig. 1).

Kihm *et al.* started by screening for genes that are turned on by GATA-1, a gene-transcription factor that regulates the production of globins and of the enzymes required to synthesize haem[10]. The screen led to the identification of one gene, expressed at relatively high levels in red blood cells, whose encoded protein — AHSP — binds specifically to α-haemoglobin *in vitro*; it does not bind to β-haemoglobin or to HbA. Kihm *et al.* also show that when α-haemoglobin is artificially overexpressed in suitable test cells, it forms large precipitates. However, when α-haemoglobin and AHSP are expressed in the same cells, both proteins remain distributed homogeneously throughout the cytoplasm. Similarly, AHSP prevents free α-haemoglobin from precipitating *in vitro*, whether spontaneously or after the oxidation of haem. Finally, like a truly enlightened chaperone, AHSP keeps a bound α-haemoglobin under control only until the latter encounters the desired partner, a β-haemoglobin.

These results could have medical implications. It was realized long ago that the balance of the α- and β-globin chains is important not just to molecular physiology but also in blood disease. Indeed, in β-thalassaemia syndromes the main defect is by definition a substantial reduction in the rate of β-chain synthesis, leading to less HbA per red blood cell. When both of the two copies of the β-globin gene are defective (that is, in the homozygous state), patients suffer from severe anaemia, which they can survive only if they receive regular blood transfusions or a bone-marrow transplant from a suitable donor[11].

In these patients, because of the lack of β-chains there is a relative excess of α-chains[12], and this is a major determinant of the severity of disease[13]. For instance, people who have a β-thalassaemia-causing mutation in just one copy of the β-globin gene (they are heterozygotes) have essentially no symptoms. However, they will develop a relatively serious condition, thalassaemia intermedia, if they also have a triplicated α-globin gene[14]. Conversely, people who would normally develop severe β-thalassaemia (thalassaemia major) as a result of a homozygous β-globin mutation can have a milder condition, again thalassaemia intermedia, if they also have mutations in the α-globin gene that reduce the concentration of the α-chain[15].

Could AHSP be relevant to these diseases? The last part of the paper by Kihm *et al.*[8] suggests that the answer might be yes. The authors engineered mice that lacked functional AHSP, and found remarkable changes in the animals' blood. The mice did not have thalassaemia, because their globin genes were intact. But their red blood cells showed abnormalities consistent with damage caused by unchaperoned α-chains. This provides *in vivo* support for the idea that the slight excess of α-chains in normal red blood cells is effectively neutralized by AHSP. By contrast, in homozygous β-thalassaemia patients, because there are no β-chains for the α-chains to pair up with, the α-chains will exceed the chaperone capacity of AHSP (the intracellular molar ratio of AHSP to α-chains is only about 1:50). The resulting damage would cause the death of maturing red blood cells.

Kihm *et al.* also speculate that naturally occurring AHSP mutations could modify the clinical picture of β-thalassaemia. There are at least two crucial tests of this idea. First, the offspring produced by mating AHSP-deficient mice with animals heterozygous for β-thalassaemia might not survive (much like mice with homozygous β-thalassaemia mutations[16]). Second, in humans, unexplained cases of thalassaemia intermedia in β-thalassaemia heterozygotes might result from mutations in AHSP that would cause the α-chain excess to be more detrimental.

The reverse situation is perhaps more difficult but is also more attractive to a haematologist. Could it be that there are mutations that result in the overexpression of AHSP, so converting thalassaemia major into thalassaemia intermedia? In every large thalassaemia centre there are rare patients, homozygous for a severe β-globin mutation, who do not depend on transfusions. Perhaps we should be studying the AHSP gene from these people first. If a mutated or overexpressed AHSP gene were so beneficial, one might be tempted to consider it for future gene therapy. In that way, a strict chaperone might become a loving nurse. ■

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Daedalus

Collimated gas

A hot gas, says Daedalus, is isotropic. You get the same temperature no matter how you hold the thermometer. If you want the gas to give some anisotropic push, you need a mechanical element — the rocket nozzle and the turbine blade are probably the best known. DREADCO chemists now want to generate anisotropic heat directly. Any fluid becomes isotropic with time, so fast reactions, explosions or rocket-reactions, seem most hopeful. Furthermore, all the energy is stored in the initial molecules. No entering oxygen complicates their anisotropic decomposition.

In this connection Daedalus recalls gunpowder, one of the cleverest of chemical inventions. At present it is made by simple mixing. The DREADCO team are devising a 'linear gunpowder' in which carbon fibres are laid alongside or interwoven with sulphur fibres (made by pouring molten sulphur into water), and these are interwoven with potassium nitrate whisker crystals. The resulting linear product, carefully aligned, should burn in the fibre-direction much faster than in the perpendicular one. Its gas will be hotter and moving faster in that direction, and colder at right-angles. The average temperature will, however, be as for normal gunpowder.

Even a small effect will be well worth having. In burning, linear gunpowder will eject an already-oriented gas. In a rocket, it will give added thrust. The rocket nozzle will have less to do, and will be burnt away more slowly by the cool sideways gas it feels. The DREADCO team also recalls how cordite is extruded as a gel in acetone — perhaps this too could be molecularly aligned anisotropically, thus gaining efficiency. The military would certainly value even a slight increase in the thrust of its rockets.

Of course, the biggest solid-fuel rockets are those of cold-war weapons and the Space Shuttle. Aligned and anisotropic fuels for these monsters could make them even more effective. On a smaller scale, the brass cartridge-case is now filled in bulk with isotropic propellant. Any ordinary gun would benefit from an oriented cartridge whose anisotropic propellant delivered its high-velocity molecules preferentially to the bullet and the breech, with less wasteful heating directed sideways at the barrel. And, of course, the delicate art of making fast- and slow-burning fuses for mining and quarrying would be greatly expanded.

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