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LETTER

The effect of sodium gold(I) thiomalate on sugar translocation across the erythrocyte membrane

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Sodium gold(I) thiomalate (Myocrisin) is one of the few compounds effective in the treatment of rheumatoid arthritis [1]. Its mode of action is as yet unclear, but it is likely that it acts via sulfydryl biochemistry [1] either by binding to thiol groups [2] or by insertion into disulfides [3]. Recent studies of the sulfydryl groups present on the extrinsic sites of proteins of the erythrocyte membrane [4] have identified these as potential binding sites for thiophyllic metals such as gold [2, 5, 6] and arsenic [7]. A possible specific binding site for gold exists on the hexose transport protein at cysteinyl residues 421 and 429 located in the proximity of helices 11 and 12 of the protein [6, 8]. The binding of gold at these sites has been investigated here to ascertain whether protein function is altered, thus identifying a new mechanism which may allow an explanation for the efficacy of gold compounds.

The rate of translocation of ions or small molecules across cell membranes can be investigated in intact cells by NMR [9, 10]. Signals from molecules such as glucose in the extracellular space can be removed from the spectrum by introduction into the extracellular medium of an inert, membrane impermeable, paramagnetic species (e.g. dysprosium tris-(2,2,6,6-tetramethyl-3,5-heptanedionate), $Dy(thd)_3$) [9]**. As a substrate traverses the membrane a signal is observed due to its presence in the cytosol [9]. Thus, using ^{13}C labelled glucose it is possible to assess the efficacy of the sugar transporter. In order to validate the method, the effect of the transport inhibitor cytochalasin B [11, 12] was also studied. This allows the response of the transporter to the presence of membrane bound gold to be clearly assessed.

To weight the experiment in favour of transport from the extracellular environment into the cell, the erythrocytes† were depleted of glucose and the cells resuspended in glucose immediately prior to measurement. Glucose transport reaches a steady state within seconds with no inhibitor present and this condition was reached before the NMR spectra were measured starting 3 min after mixing. In cell free solution β-D-glucose signals are about 10% higher than the α isomer and this is reflected in the intracellular signals. The two anomers of glucose, α and β, will slowly inter convert. However, the time required for this reaction at 25 °C is 40 min [13] and even at 37 °C the rate will be too slow to appreciably affect the amount of either anomer determined in the cell [13]. The most likely metabolites of glucose in the cell are glucose-1,6-biphosphate (66.0 ppm) and lactate (21.0 ppm). At no time are the signals from these molecules greater than 2% of the total signal. Thus, anomerisation and metabolism play only

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**An adequate $Dy(thd)_3$ concentration must be found for each batch of cells prior to glucose addition. An erythrocyte suspension was treated with trimethylammonium chloride (2 mg) which remains in the extracellular space. $Dy(thd)_3$ (2.8 mM) was added in 10 μl aliquots until the signal is nulled by > 90%. This amount of $Dy(thd)_3$ is then added to each of the suspensions prior to glucose (2.2 mg, 12 mmol) addition.

†Whole blood (20 ml) was collected in lithium heparin anticoagulant tubes. it was centrifuged at 2500 rpm for 10 min at room temperature and the buffy white coat and plasma fractions drawn off with a Pasteur pipette. The red cell pellet was then washed three times in PBS buffer (NaCl, 0.154 M; Na_2HPO_4, 0.125 M: pH adjusted to 7.4 with conc. HCl) and re-suspended in PBS (20 ml). The sample was split into four aliquots (1 ml erythrocytes:2 ml PBS). An aliquot was allowed to glucose deplete by standing for 3 h at 37 °C prior to use. An aliquot was treated with Myocrisin (65 mM) and glucose (5 mM) for 1 h to allow the protein to bind gold. The sample was then depleted of glucose by washing it three times in PBS buffer and allowing it to stand in a nutrient free buffer (2 ml) containing Myocrisin (65 mM) for 3 h at 37 °C. The third aliquot was treated with a cytochalasin B (1.4 mM)/glucose (5 mM) mixture for 1 h prior to washing and glucose depletion. The last aliquot was used immediately to establish the amount of $Dy(thd)_3$ required to efficiently null the NMR signal from the extracellular space**. Packed erythrocytes (0.4 ml) were placed in a 5 mm NMR tube with 0.1 ml of ^2H_2O/NaCl (0.154 M) to maintain a degree of fluidity within the cell suspension and provide a frequency lock for the spectrometer and 60 μl of $Dy(thd)_3$ in ^2H_2O to null the signals from the extracellular space. Spectra were recorded using a Bruker AMX-400 operating at 100.6 MHz for ^{13}C. ^1H decoupling was effected using a Waltz-16 sequence. Samples were maintained at 37 °C during data collection and the data from 1000 complete pulse sequences were accumulated for each spectrum. The free induction decay was collected in 4K of memory (acquisition time 0.819 s), zero filled to 64K. A 1 Hz line broadening function was applied prior to Fourier transformation. The 45° pulse was generated with a 4.4 μs pulse width. The spectra were obtained every 100 s and processed in absolute intensity mode.

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a minor role in this study and the subsequent results can be interpreted in terms of the separate transport of each anomer across the membrane.

In cells incubated with the competitive inhibitor, cytochalasin B, the rate of transport of both α- and β-D-glucose is decreased so that the build up of intracellular glucose can be followed (Fig. 1). The rate of uptake is significantly faster for α-D-glucose than for the β form, whereas in untreated cells β-D-glucose is transported into the cell 1.1 to 1.5 times faster than α-D-glucose [13]. β transport involves a change in protein conformation and α does not. In the presence of cytochalasin B the ordering of the transporter to facilitate β-D-glucose transport does not occur although in the absence of β-D-glucose, cytochalasin B will produce an ordering of the protein [14]. Thus, ordering of the protein is essential for efficient β-D-glucose transport.

Incubation of the cells with sodium gold(I) thiomalate produces a different effect. Compared to untreated cells there is an increase in the final concentration of β-D-glucose with respect to α-D-glucose (Fig. 1). The most likely explanation of this result is that the efflux of β-D-glucose is specifically inhibited. Efflux rates of untreated cells are known to be between 2.2 and 2.8 times faster than influx rates and there is little difference between the anomers [13]. However, β-D-glucose efflux involves a protein conformation change. The binding of gold to the two exofacial sulfydryl groups may alter protein conformation and prevent molecular recognition at the endofacial surface selectively slowing efflux. A possible role for these groups in sugar transport has been postulated for some time [15]. However, whereas cytochalasin B competes for glucose binding sites in the vicinity of helices 7 and 8 [8] preventing sugar uptake, the cysteinyl residues 421 and 429 are located in close proximity to helices 11 and 12 and are remote from the exofacial glucose binding site. This and the fact that β-D-glucose is selectively affected makes the postulate of a conformational change the most likely explanation.

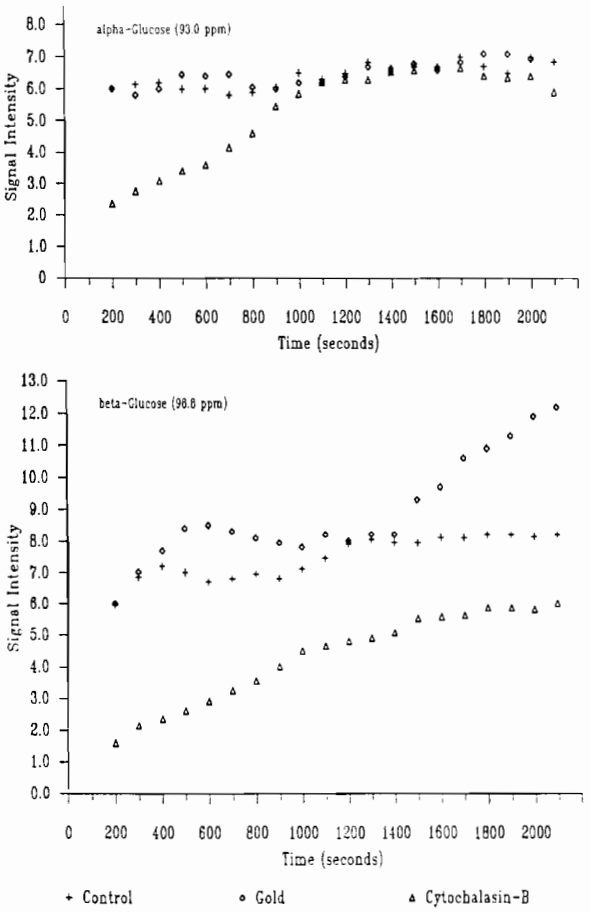

Fig. 1. Peak intensities derived from the ^{13}C NMR spectra of intact erythrocytes incubated with 2.2 mg of 99% D-glucose-1-^{13}C recorded at 100 s intervals. Bottom: β-D-glucose; top: α-D-glucose. Control (+); Myocrisin (◆); cytochalasin-B (▲).

Thus, the postulate that gold compounds affect glucose transport appears to be supported by this study of transport. In contrast to plasma and cytosolic gold where the sulfydryl levels are orders of magnitude higher than that of the gold, cell membrane gold levels reached during therapy [1] are sufficiently high for a significant number of sugar transporters to be affected. Significant changes in sugar transport could play a role in affecting cell activity and viability and hence play a part in either or both of the toxic and therapeutic actions. As such, this study would seem to indicate that the gold drug Myocrisin induces β-glucose accumulation by cells. A further interpretation of this study could be that the tetra-acetyl-thioglucose ligand in Auranofin acts as a site recognition substrate for this protein target.

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