STUDIES ON THE CONFORMATIONAL CHANGES OF METALLOPROTEINS INDUCED BY ELECTRONS IN WATER–ETHYLENEGLYCOL SOLUTIONS AT LOW TEMPERATURES. HAEMOGLOBIN*

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1. Introduction

It was shown earlier [1] that the reduction of the active centres of the haemoglobin is accompanied by considerable conformational changes involving the tertiary as well as the quaternary structure of the molecule.

Therefore, it should be expected that the reduction of the Fe-porphyrin groups of the protein in the conditions where the main conformational changes cannot take place (see [2]) leads to the appearing of the unequilibrium intermediate states with reduced active centres in the environment of the 'oxidized' structures of the protein. Studies of these states may shed light on the molecular mechanism of the reduction of the haemoglobin.

2. Materials and methods

A water solution of human oxyhaemoglobin was obtained from the Institute of Haematology and Blood Transfusions (Moscow). The oxyhaemoglobin was oxidized by potassium ferricyanide, which was later removed by gel-filtration on a G-25 column. Methaemoglobin was reduced at room temperature chemically by traces of sodium dithionite, to obtain deoxyhaemoglobin. To prevent the oxygenation of deoxyhaemoglobin, the samples were frozen rapidly at the temperature of liquid nitrogen. Protein concentrations in water–ethyleneglycol (50:50) solutions were as a rule 10^{-4} M (for measurements in Soret region -10^{-5} M). Other experimental procedures were described in [2].

It should be noted here that as in the case of cytochrome *c* the possibility of considerable radiational damages of the protein is excluded by the observation that the spectrum of deoxyhaemoglobin did not change during the irradiation.

3. Results

The spectra obtained are shown in fig. 1. As can be seen in fig.1b very great changes take place in the spectrum of methaemoglobin during irradiation. The main features of the spectrum of irradiated methaemoglobin do not coincide with the spectra of the known forms of haemoglobins, but the pattern of the spectrum allows us to assume that active centres are in the low-spin ferrous with absorption peaks at 421, (Soret band), 475, 525 (β-band) and 555 nm (α-band). Additional maxima at 625 and 404 nm are evidence of the incomplete reduction of methaemoglobin on irradiation as their intensities decrease as the dose increases. The heating of the samples of methaemoglobin reduced at low temperature, as well as of deoxyhaemoglobin (i.e. chemically reduced and frozen methaemoglobin) leads to oxygenation and the appearance of the same typical spectrum of oxyhaemoglobin. It should be noted, however, that during high doses of irradiation some irreversible destructive processes occur and formation

* The second paper in the series. The first is ref. [2].

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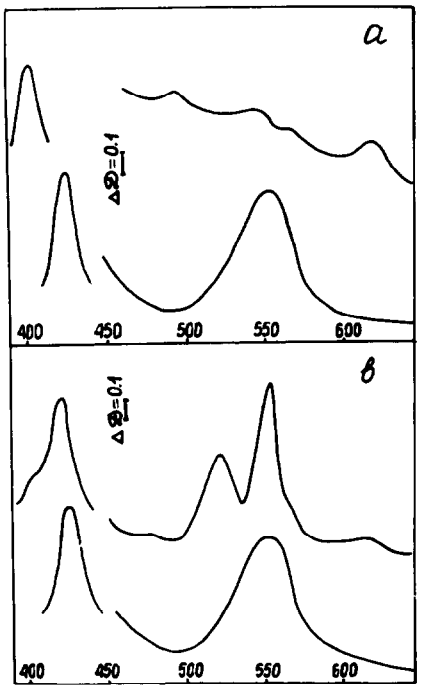

Fig. 1. Absorption spectra of methaemoglobin (Hi) (upper curve) and deoxyhaemoglobin (Hb) (lower curve) in water–ethylene glycol mixture (50:50) at 77°K before γ-irradiation (a). Spectra of haemoglobin reduced at a low temperature (upper curve) and of Hb (lower curve). Both the samples were irradiated at the same dose. (b) The curves are shifted arbitrary along the ordinate axis. Concentration of the protein in the preparations 10^{-4} M (for measurements in Soret region 10^{-5} M).

of various quantities of haemochromes can be observed on heating.

Thus, the reduction of methaemoglobin in a frozen solution by electrons generated by γ-rays leads to the formation of active centre being reduced in the low-spin ferrostate which is unusual for heamoglobin. We believe that this state of unequilibrium appears due to reduction of iron of haemoglobin in the conditions, when the structure of active centre and of whole protein can not change. After the heating the haemoglobin to room temperature, relaxation takes place and the ferrous form of haemoglobin in the equilibrium state is obtained. As the solution contains huge amounts of oxygen after irradiation on heating we observe oxyhaemoglobin instead of deoxyhaemoglobin.

4. Discussion

On the basis of the well-known crystallographic structures of haemoglobin, we propose some tentative structural explanations for the low-spin character of the spectrum. They can be divided into two groups.

1. In the hypotheses of the first group we assume that the water molecule which occupies the sixth position in the iron–porphyrin complex of methaemoglobin can move quite freely in the hydrophobic pocket of the heme even at T = 77°K. a) After reduction of the iron and departure of H_2O molecule from the sixth co-ordination position, distal histidine (E 7) forms a bond with Fe^{++} and gives rise to a hexa-co-ordinated iron–porphyrin complex where the fifth and the sixth positions are occupied by histidines. The movement of the proximal histidine (F8) does not seem to be decisive. b) As it is known the iron displaces from the heme plane on reduction [2], it is possible that in a rigid frozen solution the proximal histidine cannot move and, therefore, after departure of the H_2O molecule, a strained configuration of the active centre is formed, which has a low-spin character. Distal histidine does not form a bond with iron.

2. In the second type of hypotheses the water molecule does not leave the sixth co-ordination position of the iron. Taking into account the observations in 'inorganic' compounds [3] that systems with the electronic configurations nd^6 tend to be in the low-spin form even in relatively weak fields, we can find two possible configurations which have the spectral characteristics obtained in our experiment; strained structure with the iron in the heme plane (if the proximal histidine cannot move) and a partly relaxed one with replaced proximal histidine.

If the explanations proposed are valid and if the mobility of the active centres is comparable in the monomeric and tetrameric proteins in a frozen solution similarly 'anomalous' spectra of the intermediate unequilibrium states were to be observed in the case of myoglobin. Regrettably, myoglobin denaturated in the water solutions of 50% ethyleneglycol.

Nevertheless, a comparative study of the intermediate unequilibrium states of haemoglobin and myoglobin were carried out in the 6 M glucose water solution. The results did indeed support our supposition; the spectra of the unequilibrium state of both

the proteins correspond to the low-spin ferrous–porphyrin compounds. However, it should be noted that the vibrational structures of the α- and β-bands of the spectra of haemoglobin and myoglobin differ considerably from each other, thereby pointing to the differences in the geometry of the active centres and their nearest environments in these two cases. This, in its turn, may serve as an evidence of the existence of the different molecular pathways of the first stages of the structural changes during the reduction of the proteins. The results of these experiments will be discussed in a greater detail in the separate communication now in preparation.

5. Acknowledgement

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