Radiation Induced Structural and Functional Modification of Haemoglobin

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Abstract. An abnormality in the primary structure of dog haemoglobin was observed 1—20 days after their whole body irradiation with 190 keV X-rays (4.0 Gy). It consisted in a substitution of tryptophane residue in position 15 of the β-chain for serine. The percentage of abnormal β-chains in different time intervals after irradiation was determined. The structural changes have functional impact: an increasing haemoglobin affinity to oxygen. This could be explained on the basis of changes in the tertiary structure of haemoglobin, which may result from the substitution Trp 15 → Ser15 in the β-chain which may influence the haeme ability to bind oxygen.

Key words: Haemoglobin — Irradiation — Primary structure — Oxygen binding

Introduction

With a number of mammalian species it has been found that after irradiation with ionizing radiations some structural and functional characteristics of their haemoglobins are changed (Suchomlinov and Makovetzky 1969; Olontseva 1971; Suchomlinov and Kuznetzov 1972; Travis and Brown 1972; Suchomlinov and Kachenko 1974; Suchomlinov et al. 1981, 1982). The underlying molecular mechanisms of these events have however remained unknown. In this work the molecular nature of structural and functional modifications of haemoglobins from irradiated dogs is examined.

Materials and Methods

Dogs weighing 15—20 kg received a dose of 0.4 Gy after whole body irradiation with 190 keV X-rays (dose rate 0.05 Gy/min). Blood samples were taken prior to and on the 1st, 3rd, 6th, 12th, 15th and 20th day after irradiation, and haemoglobins were examined. Haemoglobin was obtained according to Drabkin's (1964) method and separated chromatographically on CM — cellulose ("Reanal") by the method of Peterson and Sober (1956). The separation of α- and β-chains was performed by the method of Clegg et al. (1968). Oxygen equilibrium curves were obtained spectrophotometrically by the method of Ivanov (1979). The Tyr/Trp ratio in globins was determined by a spectrophotometric method

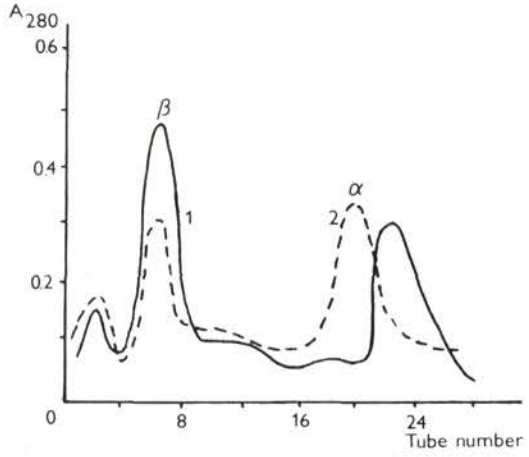

Fig. 1. Separation of α- and β-chains of haemoglobin from intact (1) and irradiated (2) dogs (on the 6th day after irradiation).

(Demchenko and Sandrovsky 1979). For globin hydrolysis "SPOFA" trypsin was used after treatment with TPCK (Carpenter 1967). Trypsin hydrolysates were analysed by the fingerprint method (Ingram 1958). Detection of histidine and tyrosine-containing peptides was performed by the method of Easley (1965), and arginine and tryptophane containing peptides by the method of Alexeenko (1968). Single peptides were isolated from the maps after staining with 0.01% ninhydrin solution. The amino acid composition of the peptides was analysed by the dansyl version of the method of Edman (Woods and Wang 1967).

Results

Haemoglobins of intact and irradiated dogs were chromatographically separated into 4 components. Their relative percentages were calculated using Gaussian areas. For intact dogs these values were: fraction I — 3.96 ± 0.87, fraction II — 1.91 ± 0.41, fraction III — 91.77 ± 0.53, fraction IV — 2.09 ± 0.5 (mean values for 10 dogs). No statistically significant deviation from the above values were observed following irradiation with one exeption. On the 15th day there was a statistically significant increase in the percentage of fraction II. A disc electrophoresis control showed that these 4 fractions were homogenous. The separation of the main component III on α- and β-chains is shown in Fig. 1.

On peptide maps of trypsin hydrolysates of haemoglobins, taken before and after irradiation, the total number of peptides was 32 : 16 in the cathodal, 12 in the neutral and 4 in the anodal zone (see Fig. 2). Dotted lines in Fig. 2 represent the ninhydrin-positive spots, stained weakly or not seen in a number of cases — presumably products of nonspecific separation with trypsin. No differences were

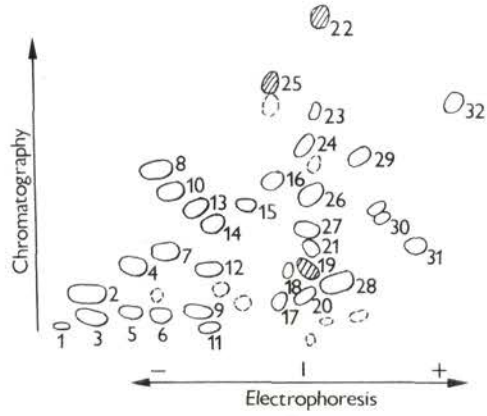

Fig. 2. Scheme of a peptide map of trypsin hydrolysate of a dog haemoglobin (IIIrd chromatographical component, intact dog). High voltage electrophoresis in pyridine-acetate buffer, pH = 5.65, during 3 hours at 35 V/cm. Chromatography in a mixture of butanol:pyridin:acetic acid:water = 15:10:3:12. *The tryptophane containing peptides are hatched.*

observed in the number of tyrosine, histidine and arginine containing peptides between the hydrolysates of haemoglobins from intact and irradiated dogs. The main qualitative difference was found for tryptophane containing peptides. Haemoglobin from intact dogs had 3 Trp-containing peptides (No 19;, 22; 25, Fig. 2). However, on the maps of trypsin hydrolysates of haemoglobins from irradiated dogs, the peptide No 25 showed no or weak Trp-positive reaction. The α-chain contains one Trp residue (in position 14), and the β-chain contains two Trp residues (in positions 15 and 37) (Dayhoff 1972). On the maps of α-chain hydrolysates (not shown) there was one peptide with Trp-positive reaction — both for intact and irradiated dogs (corresponding to No 19 on Fig. 2). On peptide maps of β-chain hydrolysates of haemoglobins from intact dogs (Fig. 3 *A*) two Trp-containing peptides were detected (corresponding to No 22 and 25 on Fig. 2). In the case of irradiated dogs (Fig. 3 *B*) the Trp-positive reaction was found only in a single peptide (corresponding to No 22 on Fig. 2), but not on the peptide, corresponding to No 25 on Fig. 2. Thus the anomalous peptide is located in the β-chain. Different mobilities of the peptides No 25 (Fig. 2) and No 16 (Fig. 3 A) may have been associated with the separation procedure of α- and β-chains. There may be some other abnormalities in haemoglobins of irradiated dogs. The present experiments were however concentrated only on the anomalous peptide No 25.

In dog β-globins, the Trp-containing peptides include the amino acid redisues from 9 to 17 and from 31 to 40. The latter also contains a Tyr residue (as the peptide No 22 on Fig. 2), Hence, the peptide No 25 contains amino acid residues from 9 to 17. The investigation of amino acid sequence showed that in the peptide

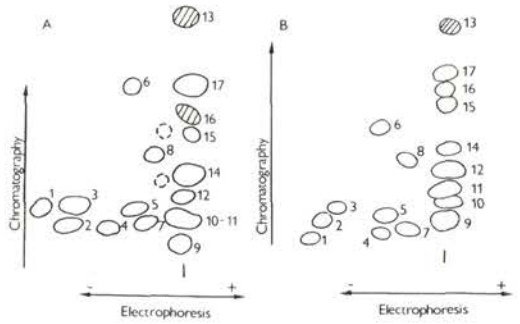

Fig. 3. Scheme of peptide maps of trypsin hydrolysates of β-globins from intact (A) and irradiated (B) dogs (on the 6th day after irradiation). For experimental conditions see the legend to Fig. 2. The tryptophane containing peptides are hatched.

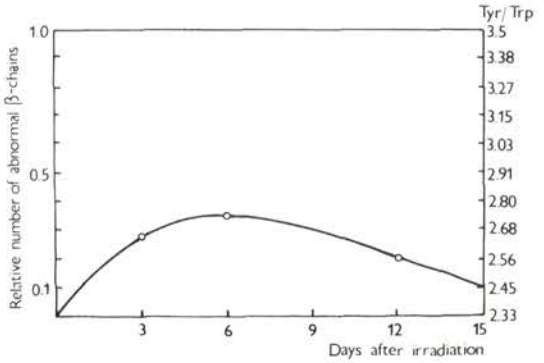

Fig. 4. The dependence of the ratio Tyr/Trp in dog haemoglobins on the time interval after irradiation.

No 25 after irradiation Trp 15 is substituted by Ser:

— Ser	— Leu	— Val	— Ser	— Gly	— Leu	— **Ser**	— Gly	— Lys —
9	10	11	12	13	14	15	16	17

The substitution Trp → Ser can take place after one transversion in the DNA codon: ACC → AGC.

To find the percentage of abnormal β-chains in dog haemoglobins at different times after irradiation we determined their ratio Tyr/Trp by means of the differential absorbtion spectra in the UV-region. The results are shown in Fig. 4. The ratio Tyr/Trp rises in the early period, reaches a maximum value on the 6th day and decreases therafter. For $\alpha + \beta$ chains of intact dog haemoglobins Tyr /Trp = 7/3. If the β-chain contains only one Trp residue then Tyr/Trp = 7/2. Taking as "x" the relative amount of the abnormal β-chains we have:

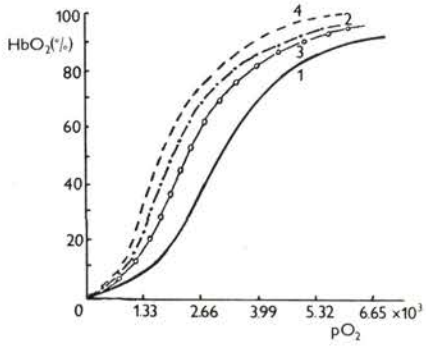

Fig. 5. The oxygen equilibrium curves of the main (IIIrd) chromatographic component of dog oxyhaemoglobins. 1 — intact dogs; 2 — on the 6th day after irradiation; 3 — on the 12th day after irradiation; 4 — on the 20th day after irradiation.

$$(7/2).x + (7/3).(1-x) = \mathrm{A} = \text{measured ratio Tyr/Trp}$$

$$\text{hence:} \qquad x = (6\mathrm{A}/7) - 2$$

The x-values are given on the left scale of Fig. 4. The maximum contribution of the abnormal β-chains, on the 6th day after irradiation, is about 35%.

From oxygen equilibrium curves (Fig. 5) of the haemoglobin main fractions (III) it is clear that haemoglobins from irradiated dogs have a higher affinity to oxygen than those from intact dogs.

Discussion

In heamoglobins the chemical surrounding of the haeme influences reversible oxygen attachment. The oxygen binding may be affected by a single amino acid substitution in the globin polypeptide chain (see e.g. Ingram 1957, 1958; Hamilton et al. 1969; Hyashi et al. 1979; Starodub et al. 1976; Veinstein and Borissov 1973). The Trp 15 residue is located in the A-segment and a occupies the position 12A (see Fig. 6). This residue is invariant for most of animal haemoglobins and is one of those which form the system of hydrophobic bonds in globine molecule (Kositsin and Ptitsin 1974). The substitution Trp 12A → Ser 12A disturbs the system of hydrophobic bonds of A-segment (Val 8A and Trp 12A) with E-segment (Phe 15E and Val 19E). The E-segment contains Val 11E, which regulates the oxygenation od the haeme. Hence we can suggest that the observed increased affinity of irradiated dog haemoglobins to oxygen is due to the substitution Trp 12A → Ser 12A in β-globins.

The described abnormities in haemoglobins of irradiated dogs may have been

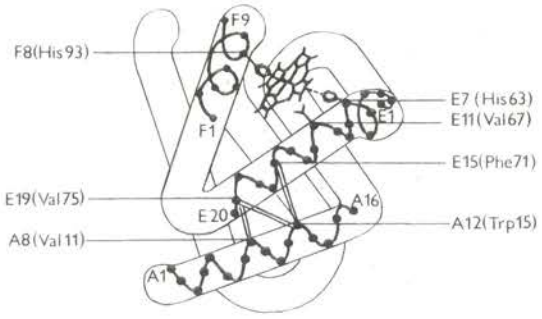

Fig. 6. Spatial organization of mammalian β-globin.

associated with different processes: somatic mutations, disturbances in the regulation of protein synthesis or with modifications of synthesised haemoglobins. These suggestions require further experimental examination.

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