ABNORMAL HUMAN HAEMOGLOBINS

II. THE CHYMOTRYPTIC DIGESTION OF THE TRYPSIN-RESISTANT "CORE" OF HAEMOGLOBINS A AND S

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The trypsin digestion of heat-denatured haemoglobins A and S left a dark brown insoluble material which contained the haeme groups and a peptide or peptides[1]. Chymotryptic hydrolysis digested this further to a soluble mixture of peptides which was then examined by fingerprinting and chromatography.

Heat-denatured haemoglobins A and S were digested with trypsin as described in the preceding paper. The residue from only one sample of each haemoglobin was examined, though the trypsin and chymotrypsin digestions were carried out twice. Immediately after trypsin digestion, the pH of the mixture was lowered from 8 to 6.5 with HCl and the brown precipitate, carrying most of the colour, was removed by centrifugation. This is either washed at once or frozen and thawed to form a dense precipitate which is easier to wash and does not disperse. The washing was done by centrifugation and comprised three cycles with 20 volumes of dilute pyridine–acetic acid–water buffer, 2:0.08:97 by volume, pH 6.4, followed by three washes with water. The precipitate was dissolved in 1–2 volumes of cold 0.1 *N* HCl and was then treated with 10 volumes of ice-cold acetone containing 1 % of 1 *N* HCl by volume, followed by

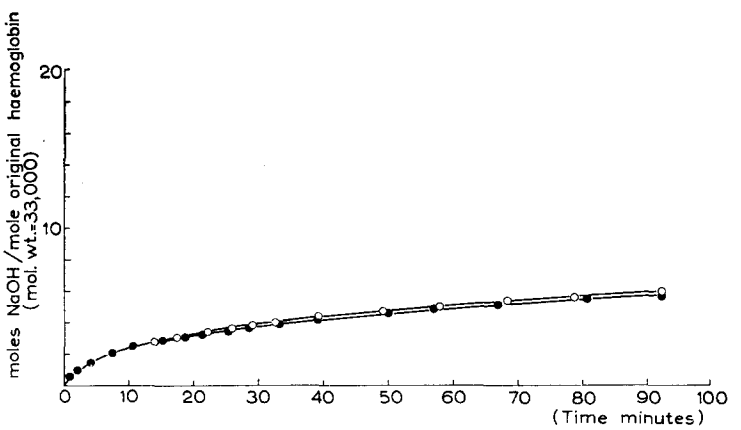

Fig. 1. Course of the chymotrypsin digestion of the "cores", from haemoglobins A and S. ○---○ Haemoglobin A; ●—● Haemoglobin S.

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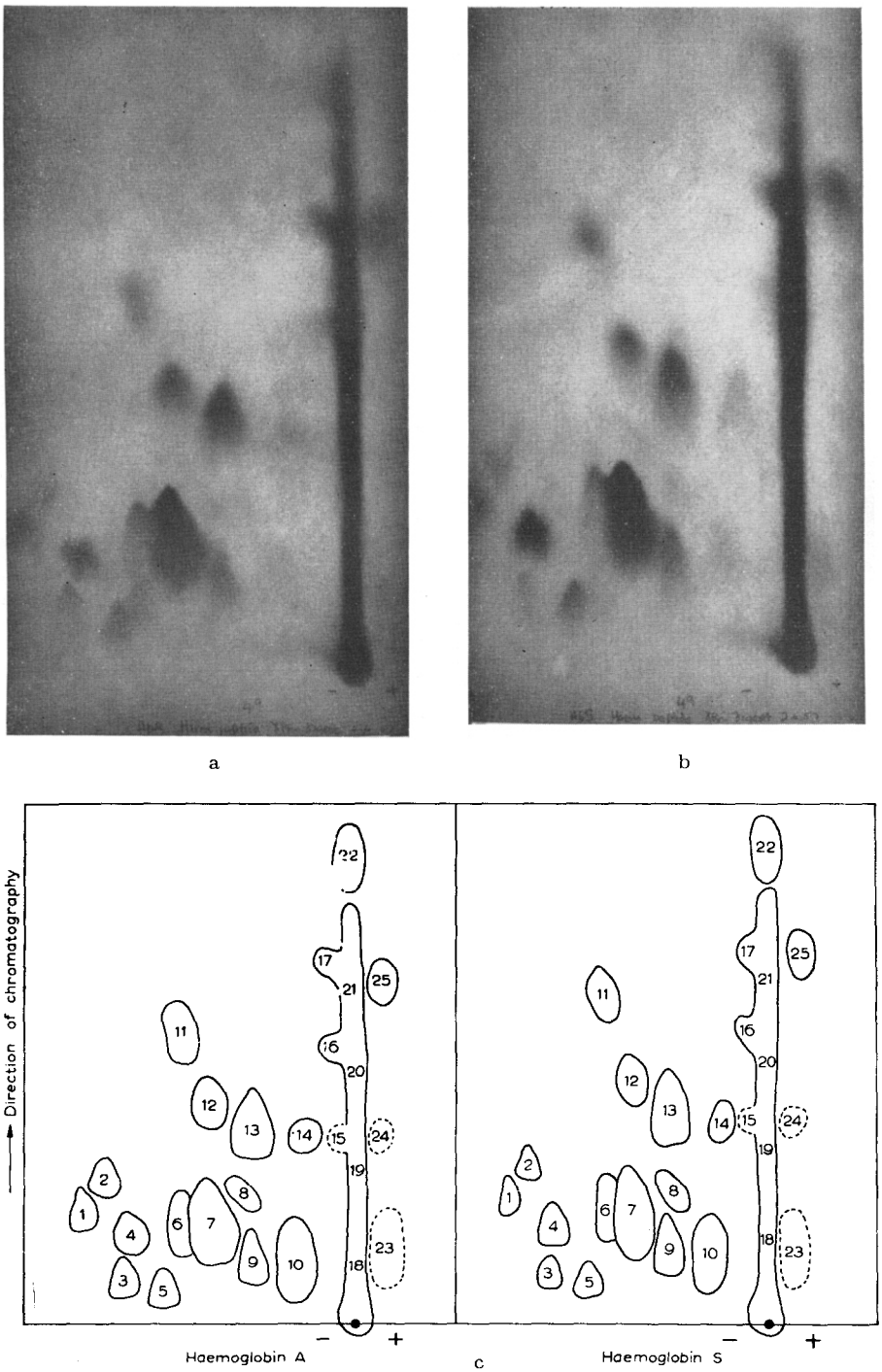

Fig. 2. Fingerprints of chymotrypsin digests of "cores" from haemoglobin A and S. a. Haemoglobin A; b. Haemoglobin S; c. Tracing. Dotted lines indicate spots visible after heating.

pure acetone. Vigorous shaking brought most of the haeme colour into solution and precipitated the peptide in floccules. A little more precipitate could be obtained by adding an equal volume of acetone to the supernatant solution. The peptide, very light brown in colour, from 88 mg haemoglobin was dissolved in 3 ml of water and traces of acetone were removed by placing the solution in a vacuum desiccator for a short while. The volume was made up to 4 ml and the peptide was ready for digestion in the apparatus already described. The pH was raised to 8.0; the solution remained clear or became cloudy, but became clear during the digestion.

Chymotrypsin had been prepared and crystallised from dilute alcohol according to NORTHROP AND KUNITZ[2]. The amount of enzyme added to the "core" peptide derived from 88 mg of haemoglobin was the same as the amount of trypsin used originally. The chymotrypsin digestion was allowed to proceed at 38° at pH 8.0 for about 90 min, keeping the pH constant within 0.05 pH units by addition of $M/4$ NaOH; by this time the uptake of alkali had reached a constant slow value (Fig. 1). The curves for the two haemoglobins were closely similar. The number of peptide bonds broken was around 5–6 per 34,000 mol. wt. of original haemoglobin which indicated on the one hand good agreement with the six or so strong peptides formed after this digestion and on the other hand that the yields in the isolation of this peptide must be good. The digestion was stopped by freezing.

The peptides obtained from haemoglobins A and S "haeme peptides" were fingerprinted by the usual method, after concentrating 0.50 ml to 0.040 ml and then using 0.020 ml for one fingerprint. Good fingerprints were obtained which corresponded in every detail (Fig. 2). However, the band of apparently neutral peptides was so badly adsorbed that they had to be resolved separately.

For this purpose 1 ml of each chymotrypsin digest originally from haemoglobins A and S was evaporated, dissolved in 0.080 ml of water and applied to the next paper as a 4″ strip. Electrophoresis on 3MM paper in one dimension in the pH 6.4 buffer at 20 V/cm gave the neutral peptides as a band which was eluted with 20% acetic acid. The peptides were examined by cooled paper electrophoresis on 3 MM paper in the pH 3.6 buffer at 35 V/cm for 80 min. No difference was detectable between the peptides derived from the two haemoglobins (Fig. 3). The neutral peptides were also analysed by descending chromatography on Whatman No. 1 paper in *n*-butanol–acetic acid–water, 4:1:5. Here also, there were no detectable differences (Fig. 4).

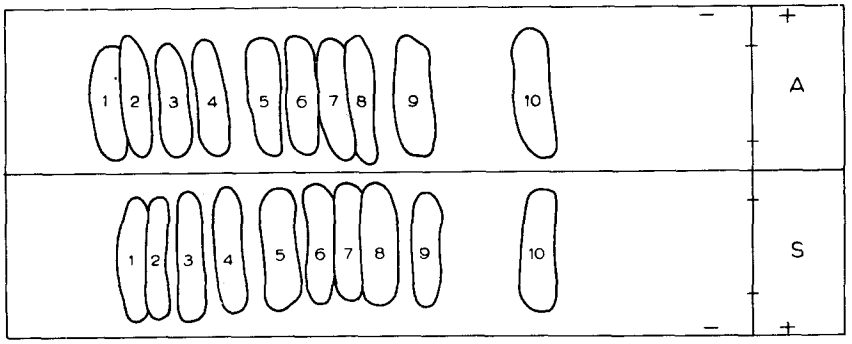

Fig. 3. Tracing of paper electrophoresis at pH 3.6 of neutral peptides from chymotrypsin digestion of haemoglobin "cores".

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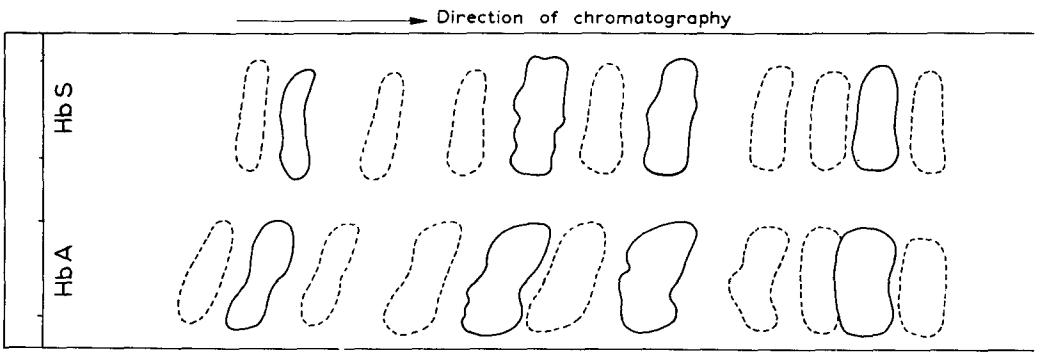

Fig. 4. Tracing of chromatogram of neutral peptides from chymotryptic digestion of haemoglobin "cores". Solvent: *n*-butanol–acetic acid–water, 4:1:5.

All individual spots from six fingerprints each of the haemoglobins A and S "core" digests were eluted, hydrolysed as described earlier, and their constituent amino acids investigated by paper chromatography in the REDFIELD system[3]. The limitations of this method have been discussed above. Apart from these, it was not possible to demonstrate a difference between any homologous peptide pairs; the apparently neutral peptides were examined separately by successive electrophoreses at pH 6.4 and pH 3.6 (Fig. 3). Each band was analysed but no differences in amino acid composition could be found. These studies also indicated that the fingerprints contained in all probability only six significant peptides, at least two of which behaved as though they were large. It is likely that some of the others are either small amounts of absorbed trypsin peptides or degradation products of these. For example peptide 11 appears to be the same as trypsin peptide No. 18. It is remarkable that in spite of such adsorption extremely reproducible fingerprints are obtained.

The conclusion of these studies is that the so-called "trypsin-resistant cores" of haemoglobins A and S account for a considerable, though unknown, portion of the haemoglobin molecule. Apparently this is a region poor in lysine and arginine, but quite rich in the aromatic amino acids and those with the long non-polar side chains. Careful comparison of chymotryptic digests of these two "cores" failed to detect any differences between them. This strengthens the conclusion of the first paper that the only amino acid difference between haemoglobin A and S lies in the No. 4 peptides of the trypsin digests.

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SUMMARY

No differences were detected between the "trypsin-resistant cores" of haemoglobins A and S. The core accounts for a considerable portion of the molecule, poor in lysine and arginine, but rich in aromatic amino acids and those with the long non-polar side-chains.

REFERENCES

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[3] R. R. REDFIELD, *Biochim. Biophys. Acta*, 10 (1953) 344.

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