COMPARATIVE INVESTIGATION OF MICROHETEROGENEITY OF MYOGLOBINS IN SOME ANIMALS AND MAN

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During the investigation of human, equine, bovine, and whale myoglobins by starch-gel electrophoresis considerable differences were found in their mobility and microheterogeneity of all the tested myoglobins was discovered. During investigation of equine myoglobins by continuous paper electrophoresis 5 components were found.

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Previous papers [2-4] described the results of a comparative investigation of equine, bovine, human, and whale myoglobins by the peptide map method. Crystalline myoglobins are known to possess microheterogeneity [8].

This paper describes a comparative investigation of microheterogeneity of equine, bovine, human, and whale myoglobins by starch-gel electrophoresis and fractionation of equine myoglobin by continuous paper electrophoresis.

EXPERIMENTAL METHOD

The myoglobins were isolated as described previously [2]. All myoglobins were obtained in the crystalline form.

Starch-gel electrophoresis was carried out by Smithies' method [10, 12] in tris-citrate buffer, pH 8.6, for 4 h (voltage 400 V, current 10-20 mA, temperature 4-5°). The gel was stained with a 2.5% solution of amido black. After staining the gel was washed with a methanol — acetic acid — water (3 : 5 : 1) mixture.

Continuous electrophoresis was carried out on the apparatus constructed by Mant'ev [1] on slowly absorbing chromatographic paper (Leningrad) in veronal buffer (pH 8.6, ionic strength 0.015) for 64-66 h at 3-4° (voltage 800-1000 V, current 2-3 mA). A 2.5% solution of myoglobin was used for electrophoresis.

Electrophoresis in a Tiselius apparatus was carried out in 1.5 M phosphate buffer, pH 8.0, with a current of 17 mA for 3-4 h.

Analytical ultracentrifugation was carried out for 4 h in citrate buffer, pH 5.8, with a rotor speed of 61,000 rpm at 22°.

N-terminal groups were determined by Sanger's dinitrofluorobenzene method [11].

EXPERIMENTAL RESULTS

The results of starch-gel electrophoresis of equine, bovine, human, and whale myoglobins are given in Fig. 1. It is clear from Fig. 1 that the investigated proteins differ significantly in their electrophoretic mobility. During starch-gel electrophoresis bovine myoglobin moved more rapidly than the rest, human and equine myoglobins somewhat more slowly, and with almost identical electrophoretic mobility, while whale myoglobin moved the slowest of all, its principal component remaining almost at the starting line. All myoglobins investigated by starch-gel electrophoresis (Fig. 1) separated into several components (one principal and several minor), the principal component not succeeding in dividing into two before the end and moving more slowly than the minor components.

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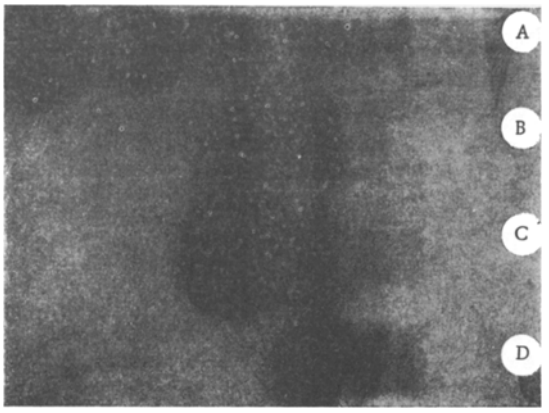

Fig. 1. Electrophoresis of whale (A), human (B), equine (C), and bovine (D) myoglobins. Starch-gel electrophoresis in tris-citrate buffer, pH 8.6 400 V, 10-20 mA.

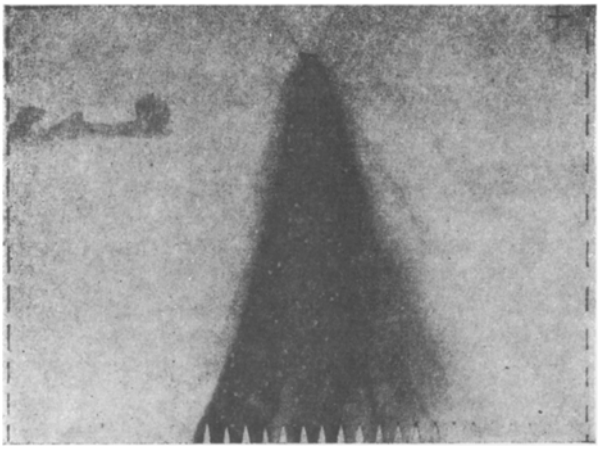

Fig. 2. Electrophoresis of equine myoglobin. Continuous electrophoresis in veronal buffer, pH 8.6, μ=0.015, 1000 V, 2.9 mA. Duration of electrophoresis 66 h.

Equine myoglobin was also investigated by continuous paper electrophoresis. As Fig. 2 shows, equine myoglobin divided into 5 components. Two components, constituting the main mass of the myoglobin, had not fully separated by the end of electrophoresis and showed only slight relative displacement of their stains. The remaining three components, with a higher negative charge, were minor components of myoglobin. On the basis of earlier results [2] obtained by chromatography of equine myoglobin on a column of Amberlite IRC-50, it can be postulated that the principal components of equine myoglobin during continuous electrophoresis correspond to the principal peak during chromatography on the Amberlite IRC-50 column, and the remaining three components correspond to the minor peaks. Traces of other components demonstrated by continuous electrophoresis evidently were due to very small amounts of impurities in the preparation. Sedimentation analysis of the equine myoglobin in the ultracentrifuge gave only one peak, and determination of N-terminal amino acids by the dinitrofluorobenzene method revealed only one N-terminal amino acid to be present, namely glycine. Electrophoresis of the myoglobin in a Tiselius apparatus gave only one, very asymmetrical peak.

Microheterogeneity of the myoglobin was discovered to a varied degree by the use of different electrophoretic and ion-exchange chromatographic methods, but not by ultracentrifugation nor by determination of N-terminal groups. Differences between the individual components of the myoglobins are evidently due to differences in their charges and not to differences in their molecular weight or terminal groups. These results are in agreement with those obtained by other workers. Akeson and Theorell [5, 6], for example, separated equine myoglobin on a CM-cellulose column into three components and found no differences between these components either in molecular weight or in terminal amino acids.

The results obtained by starch-gel electrophoresis were then compared with results previously obtained using the peptide map method for myoglobins of the same species of animals [4]. Investigation of trypsin and chymotrypsin hydrolysates of equine, bovine, human, and whale myoglobins showed that bovine and whale myoglobins possess the smallest number of identical peptides, obtained with both trypsin and chymotrypsin. These proteins also show the greatest differences in mobility during starch-gel electrophoresis. Equine and human myoglobins were closest in their behavior during starch-gel electrophoresis. They were also the closest as regards the number of identical chymotrypsin peptides. As regards the number of identical trypsin peptides, equine and whale myoglobins showed the closest resemblance. However, the results obtained on peptide maps of chymotrypsin hydrolysates reflected the structure of the myoglobin molecule more completely than the results obtained on trypsin hydrolysates, because trypsin does not split the whole myoglobin molecule. Hudgins and co-workers [9], who investigated the myoglobins of various species of primates, also found a correlation between the results of starch-gel electrophoresis and the peptide composition of enzyme hydrolysates of the myoglobins.

Bernard and co-workers [7] investigated equine, bovine, ovine, and porcine myoglobins by starch-gel electrophoresis and found that equine, bovine, and ovine myoglobins move at the same velocity, and

porcine myoglobin somewhat slower. The present experiments revealed considerable differences in the velocity of movement of equine and bovine myoglobins during starch-gel electrophoresis, whereas Bernard and co-workers [7] found no such difference. This can presumably be attributed to the rather different conditions of electrophoresis in the present experiments.

By starch-gel electrophoresis we demonstrated species differences in mobility between human, equine, bovine, and whale myoglobins and demonstrated microheterogeneity of the investigated myoglobins. During starch-gel electrophoresis we found not more than four components of the myoglobins, whereas, by continuous paper electrophoresis we found five distinct components, presumably because of the greater resolving power of continuous paper electrophoresis.

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