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Some Hybrids of Deoxygenated Sea Lamprey Hemoglobins (*Petromyzon marinus*)

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Oxy-hemoglobin from the sea lamprey is a single chain, single heme molecule (Mol. wt. 18 400). Even though there are six different lamprey hemoglobins they only associate when deoxygenated. Upon mixing the six hemoglobins with each other, and deoxygenation it was possible to form several hybrids. It appears that the various monomers have preferential affinity for each other. The electrophoretic mobility of the hybrid was always governed by that of the faster component. Despite the wide variety of hybrids possible, only two bands appear on gel electrophoresis; however, it is believed these are not homogeneous but consist of several hybrids with nearly similar mobilities.

In a previous paper, the isolation of the six lamprey hemoglobins was reported[1]. It was also previously found that deoxygenated lamprey hemoglobin forms dimers and tetramers[2]. Although these experiments had been done in the analytic ultracentrifuge where hybrid dimers and tetramers would be indistinguishable from self-dimers and self-tetramers, it was possible to show the formation of hybrids (Hb_1Hb_2), (Hb_1Hb_4), (Hb_1Hb_5), ($Hb_3Hb_4)_2$ and ($Hb_3Hb_5)_2$[2]. Thus a preferential affinity of the various deoxygenated monomers for each other is indicated.

This paper reports some further experiments on the formation of hybrid polymers, as revealed by anaerobic electrophoresis on a gel of cyanogum 41.

PREPARATION OF HYBRID HEMOGLOBINS

The six lamprey hemoglobins were separated and purified by continuous flow paper electrophoresis[1]. The homogeneity of each component was checked by free boundary electrophoresis at three different values of pH[1]. The homogeneous components were dialyzed salt-free against distilled water plus a drop of ammonia to keep the pH between 7.0 and 7.5. After determination of the concentration by dry weight, the salt-free material was divided into portions of 0.3 ml and kept frozen for a few days, until needed.

The individual hemoglobins were thawed, mixed in depression slides, refrozen and introduced into a glove box. This was flushed with a mixture of 99 % prepurified N_2 (99.97 % pure) and 1 % H_2 which was passed over hot copper. The glove box also had a piece of hot copper

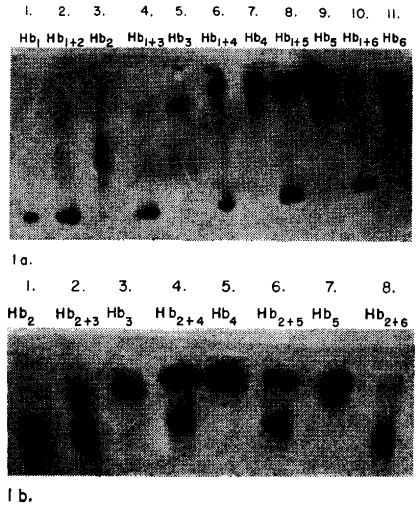

Fig. 1. a) Cyanogum 41 gel electrophoresis of deoxygenated hemoglobin 1 and equimolar mixtures of Hb_1 with the other five Hb's. b) The same for hemoglobin 2.

in it, and the atmosphere inside was stirred with a fan so that any oxygen coming in would be eliminated.

The cyanogum 41 gel was made by mixing 300 ml of tris buffer[3] pH = 7.6, after it had been degassed with a water pump for 15 min, with 30 g of cyanogum 41 (American Cyanamid Co.) and 56 ml of 10 % DMAPN (dimethylaminopropionitrile) which was also degassed. Finally, 3.6 ml of 10 % ammonium persulphate and 300 mg of sodium dithionite were mixed in, and the solution was poured into a lucite frame 37 × 14.5 × 0.4 cm. The degassing seems to inhibit gel formation and it was found necessary to add 1.5 ml more of the ammonium persulphate solution to produce gelation.

The slits were made in the gel with a razor blade and pieces of Whatman 3 MM filter paper which had been dipped into the tris buffer and dithionite were inserted into the slits. The gel block was then covered with saran film and placed in the glove box on a cooling plate circulated with water at 4°C, and left there for a few hours.

Inside the glove box the ferri-hemoglobin mixtures were reduced with sodium dithionite and were transferred into the pre-cut gel with pieces of filter paper. Cloth wicks were used to connect the gel block to the electrode vessels which contained 0.3 M borate buffer[3,4]. About 160 V and 105 mA were applied for about six hours. After the run the gel block was removed from the glove box and developed with buffalo black. The excess dye was washed off with Smithie's solution[3].

Alkaline pyrogallol was used to check for oxygen in the glove box.

RESULTS

Despite the above precautions, it is not certain that completely anaerobic conditions were obtained. Nevertheless some preliminary observations on the formation of hybrids are worthy of note. Figs. 1a and b show the gel electrophoresis of hemoglobins 1 and 2 in all possible equimolar combinations with the other four hemoglobins. Figure 2 shows combinations of hemoglobins 3, 4, 5 and 6. Figure 3 shows unpurified ferri-hemoglobin (aerobic conditions), the same sample, reduced (anaerobic), the same sample enriched with hemoglobins

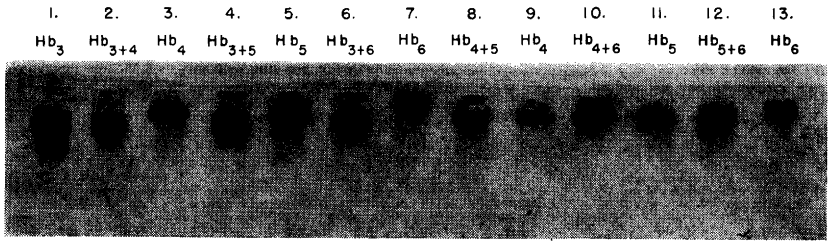

Fig. 2. Cyanogum 41 gel electrophoresis of deoxygenated hemoglobins 3, 4, 5 and 6 and their equimolar mixtures.

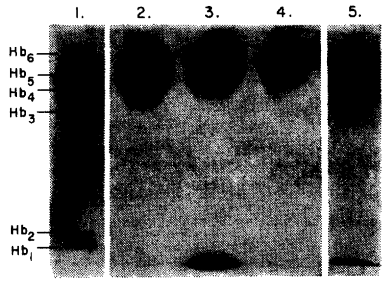

Fig. 3. Cyanogum 41 gel electrophoresis. 1) Unpurified ferri hemoglobins (aerobic). Hemoglobins 4 and 5 were not resolved under these conditions. 2) Same mixture as 1) but reduced and anaerobic. 3) Same as 2) but enriched with hemoglobins 1 and 2. 4) Same as 2) but enriched with hemoglobin 3. 5) Equimolar mixture of all six hemoglobins, reduced and anaerobic.

1 and 2, reduced (anaerobic), the same sample enriched with hemoglobin 3, reduced (anaerobic), and an equimolar mixture of all six hemoglobins, reduced (anaerobic).

DISCUSSION

From the results obtained thus far it is believed that hemoglobins 1 and 2 only form dimers in equimolar mixtures with the other hemoglobins. It appears that the hybrids of hemoglobins 1 and 2 with the others are easily dissociated, and therefore frequently show two electrophoretic bands in these mixtures. Indeed, in the gel experiment it is not at all clear that hemoglobin 2 forms hybrids, but the ultracentrifuge clearly shows a hybrid (Hb_1Hb_2) dimer[2].

The experiments with unpurified hemoglobin and with the equimolar mixture of all six show only two electrophoretic bands. We suspect that the slow band is not homogeneous, but consists of several hybrid tetramers which did not separate due to their very similar mobilities. The fast band, which in the standard mixture is very faint, probably consists of hybrid dimers of hemoglobins 1 and 2 with the others. Further work is necessary to identify the components present and their ratios. In the case of the equimolar mixture, it is quite clear that all possible hybrids are not formed and therefore the affinity of the deoxygenated monomers for each other is preferential. On the basis of the above experiments, ultracentrifuge experiments[2] and some speculation we believe equimolar mixtures will behave as shown in Figure 4.

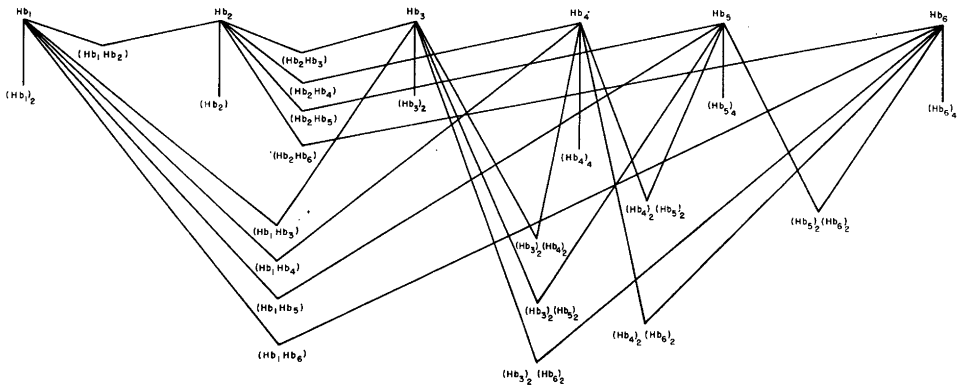

Fig. 4. The proposed behavior of the six deoxygenated lamprey hemoglobins when mixed 1 to 1.

It may be that hemoglobins 1 and 2 are evolutionary relics, and that the development of a tetramer-forming capacity conferred a physiological advantage upon the lamprey. It is certainly true that the oxygenation of a dimer or tetramer, whether or not it dissociates afterward, presents the possibility for heme-heme interaction, or sigmoid dissociation curves with their well-known advantages.

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