FUNCTIONAL ABNORMALITY OF HEMOGLOBIN M_{OSAKA}

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A number of reports on the relationship between the structure and the function of hemoglobin have been published. However, few studies (Benesch, R.E. et al., 1961; Horton, B. F. et al., 1962) have been made on the functional properties of abnormal hemoglobin, although such a study may be expected to furnish a valuable clue in understanding the structural basis of oxygen carriage function of hemoglobin.

In 1963 the authors detected a new variant of hemoglobin M in a Japanese family living in Osaka, Japan, and designated it as hemoglobin M_{Osaka} (Hb M_0) (Hayashi, A. et al., 1964). As a result of chemical analysis, the primary structure of Hb M_0 was determined to be $\alpha_2{}^{58Tyr}\beta_2{}^{A}$ (Shimizu, A. et al., 1965). The present communication describes the functional properties of Hb M_0 isolated from the blood of the propositus.

The separation of Hb M_0 was carried out chromatographically, as stated in the previous paper (Shimizu, A. et al., 1965). For the experiments the hemoglobin in stock was adjusted to about 6×10^{-4}M and equilibrated with 0.2 M potassium phosphate buffer of different pH values. The mixture was then centrifuged at 8,000 r.p.m. for 20 minutes and the clear

supernatant was transferred into a cell-fused tonometer (0.2 cm light path). The oxygen equilibrium curves were determined at 19.5°C by the method of Allen and Wyman with minor modifications (Enoki, Y., 1959). The percentage of oxygenated Hb was computed from the optical density change on oxygenation at around 700 mμ. In experiments with both Hb M_0 and Hb A air was used to attain the oxygenation, but in later stage of the study it became apparent that Hb M_0 was not completely saturated at the atmospheric pressure. So the data for oxygen equilibria of Hb M_0 were corrected by the experiments in which it was further equilibrated with atmospheric oxygen instead of air. After each experiment the oxygen capacity, pH and hemoglobin concentration were measured.

The resultant oxygen equilibrium curves are presented in Figure 1.

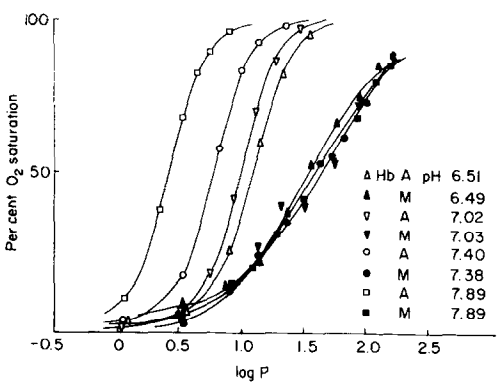

Fig. 1. Oxygen equillibrium curves of the Hb M_{Osaka} and Hb A fraction from Hb M_{Osaka} patient.
Hb concentration in 0.2 M Potassium Phosphate buffer; about 2.4×10^{-3}M as heme.

In the first place, it is obvious that the position of the curves of Hb M_0 are unusually shifted to the right: in other words the oxygen affinity of Hb M_0 was very low, e.g. at pH 7.4 nearly one seventh of that of Hb A.

Secondly, the abnormal shape of the curves of Hb M_0 is worthy of note. The values of n in Hill's empirical equation, a numerical expression of the intensity of heme-heme interaction, were above 2.6 for Hb A and 1.2 or so for Hb M_0 (Figure 2).

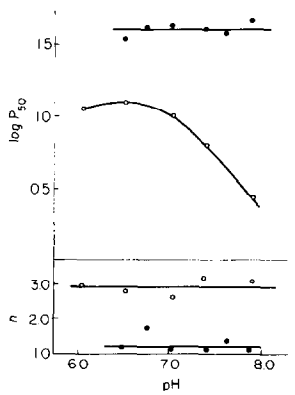

Fig. 2. Oxygen affinity and shape of the oxygen equilibrium curves of Hb M and Hb A fraction at various pH.
o = Hb A ● = Hb M_{Osaka}

And the third characteristic of Hb M_0 is that the Bohr effect i.e. the effect of pH on the oxygen equilibria was almost unrecognized (Figure 2).

In Hb M_0 it is supposed that an internal complex is formed between the heme iron and the side chain of the tyrosine which substitutes the histidine (Gerald, P.S. and Efron, M.L., 1961). This heme iron persists in ferric state even in red cell where a powerful methemoglobin reducing mechanism is in action. Loss of sigmoid character of the curves which we observed may be interpreted as follows: one possibility is that it may be derived from the peculiar state of the heme iron, for a similar behavior of the curves has been observed

in Hb A some irons of which are rendered ferric artificially (Darling, R.C. and Roughton, J.W., 1942). A second possibility is that Hb M_0 may be a functional dimer of β-subunits as in Hb H, a tetramer of β-subunits (Benesch, R.E. et al., 1961). Neither of the possible mechanisms, however, can explain the unusual low level of the oxygen affinity of Hb M_0, because both the partially oxidized Hb A and the Hb H showed on the contrary a very high oxygen affinity.

Therefore a hypothesis might be presented here that the abnormal α-chain of Hb M_0 interrupts the oxygenation of β-chain, with the following mechanism: the formation of an internal complex between the heme iron and the side chain of tyrosine gives rise to the helical distortion and further the modification of the folding of α-chain, which in turn alter both the folding and the arrangement of β-chain so that the latter chain can not easily attain the obligatory conformational change on oxygenation. This may be followed by the absence of the Bohr effect. Recently Kikuchi et al. (1964) in the study on Hb M_{Iwate} also reported the similar results to Hb M_0, except the existence of a slight Bohr effect above pH 7.5.

However the mechanism almost remains unknown in detail. Further experiments are in progress to make clear these interesting problems.

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