THE SUBUNIT STRUCTURE OF HAPTOGLOBINS.

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In a previous paper from this laboratory (M. Waks and A. Alfsen, 1966), the structure of haptoglobins, (Hp 1-1 and Hp 2-2) the hemoglobin-binding proteins of human serum, has been examinated. The hydrogen ion titration curves, the tyrosine spectrophotometric titrations and optical rotatory dispersion gave very close data for both genetic species, and suggested a similar subunit structure.

The present report describes the behaviour of haptoglobins in the alkaline pH range. The molecules undergo a reversible dissociation equilibrium : Hp 1-1 between pH 9.0 and 11.5, Hp 2-2 between pH 9.0 and 12.0. At pH 11.50, the ultracentrifuge data indicate that haptoglobins are dissociated, homogenous and contain a subunit of identical molecular weight of 42.000 ± 3.000.

Material and methods : Pure haptoglobin of genetic type 1-1 and 2-2 was prepared as previously described (Waks and Alfsen 1966). All other reagents were the best available com-

mercial products and were used without further purification.

Sedimentation velocity experiments were performed in a Spinco Model E Ultracentrifuge equipped with phase plate schlieren optics and a rotor temperature control unit. The S values were not corrected for the viscosity of the protein. The sedimentation equilibrium experiments were performed as described by Yphantis (1964). The value of the apparent partial specific volume, calculated from the amino-acid and sugar composition, (Cloarec and al 1963) was 0.710 for Hp 1-1 and Hp 2-2.

Results : At 20°C, in 0.3 M KCl, from pH 5.5 to pH 9.5, Hp 1-1 sediments as a symmetrical peak, giving a sedimentation coefficient of 4.10; Hp 2-2, in contrast, displays a heterogenous pattern with at least two components. The sedimentation coefficient of the slow component was calculated to be 3.5 and that of the fast 7.5 (fig. 1). It is clear from fig. 2, that, in the pH range 9.5 - 12.0, the sedimentation coefficient of Hp 1-1 decreases with increasing pH. The curve obtained

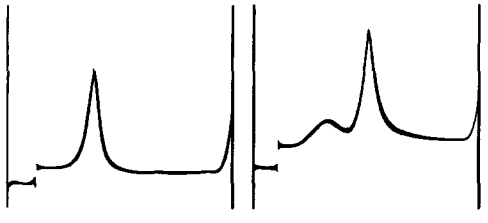

Fig. 1

Sedimentation velocity patterns of 0.6% Hp 1-1 (left) and Hp 2-2 (right) in 0.3 M KCl, pH 5.50, at 20°C. Pictures were taken 50 min. after attaining the speed of 59,780 rpm.

by plotting S app. against pH (fig. 2) is completely reversible between pH 9.5 and 11.5. At higher pH values, an irreversible transformation occurs. This irreversible change is prevented by addition of iodacetamide to the protein solution at pH 8.5. In these conditions the S value does not decrease further than 2.9.

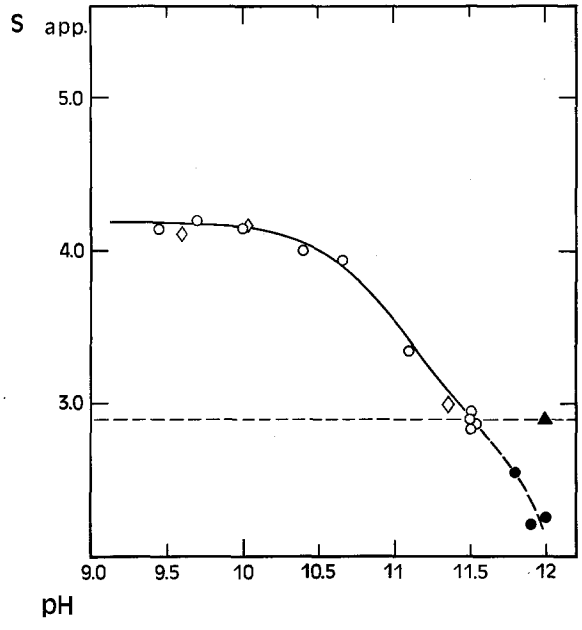

Fig. 2
Variation in the apparent sedimentation coefficient of 0.5% Hp 1-1 as a function of pH. Solid circles represent the irreversible part of the curve. Diamonds, back titration data. Solid triangles : experiments in presence of iodacetamide.

The variation of S app. for 0.5% Hp 2-2 with increasing pH values, is illustrated by the data in table I. A fast transition occurs between pH 11.3 and 11.5 and a single 2.9 S peak (fig. 3) is observed between pH 11.5 and pH 12.0. This transition is completely reversible : i.e. at any pH values between 12.0 and 11.5 the back titration of Hp 2-2 shows the

same two peak schlieren pattern observed in fig. 1, with unchanged sedimentation values of 7.1 S and 3.5 S.

TABLE I

pH	S app.		
9.00		3.5	7.1
10.80		3.5	6.5
11.30		3.5	6.6
11.50	2.9		
11.80	2.8		
12.00	2.9		

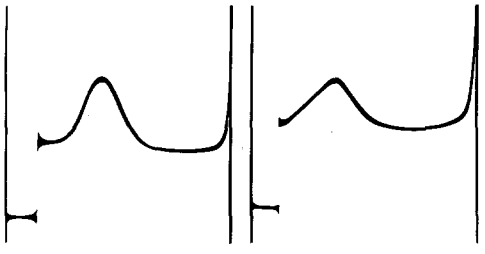

Fig. 3

Sedimentation velocity patterns of 0.5% Hp 1-1 (left) and Hp 2-2 (right) at 20°C in 0.3 M KCl-KOH, pH 11.50. Pictures were taken 92 min. and 82 min. after reaching a rotor speed of 59,780 rpm

The symmetrical peak observed throughout the course of the experiment suggests a relative homogenous species.

The molecular weight of the 2.9 S sedimenting species was determined by sedimentation equilibrium experiments. The plot of ln c versus x^2 for Hp 1-1 and Hp 2-2 give a straight line (fig. 4 and 5). The molecular weight calculated from the slope is 42.000 $\pm$ 3.000 for Hp 1-1 and Hp 2-2.

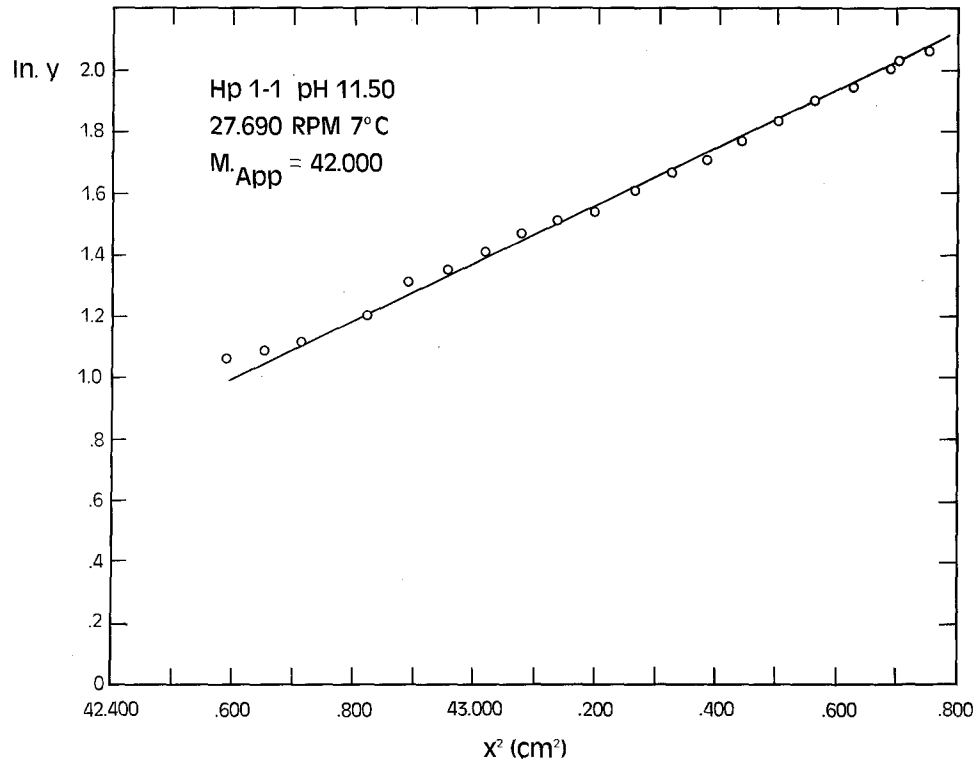

Fig. 4 : Molecular weight determination from sedimentation equilibrium experiments on 0.03 % Hp 1-1 at pH 11.50 in 0.3 M KCl-KOH. Plot of the logarithm of protein concentration as a function of the square of the distance from the center of rotation.

Discussion : The data show that both genetic types of haptoglobin undergo reversible dissociation at alkaline pH. The possibility of a change in molecular shape, by unfolding, which could produce a corresponding decrease in the sedimentation coefficient, can be discarded since the molecular weight of the subunit, is approximately one half that of Hp 1-1 at neutral pH (M.W. = 85.000). It appears that the ionisation of some charged groups is the determining feature

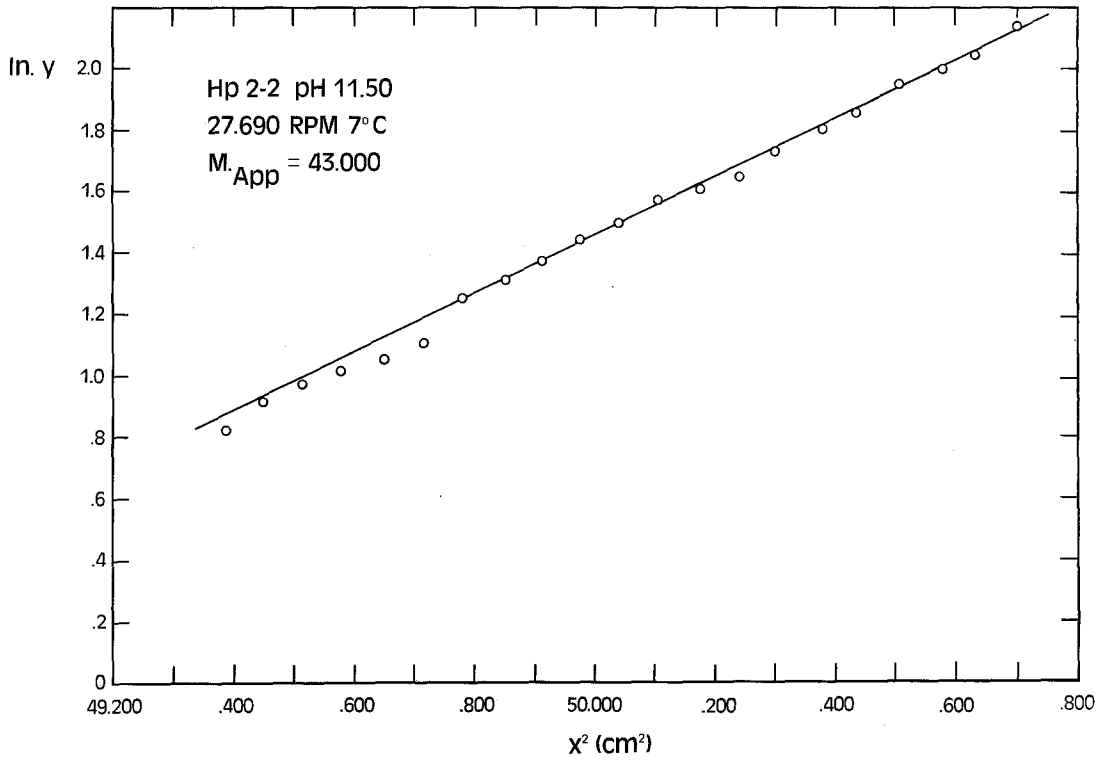

Fig. 5 : Molecular weight determination from sedimentation equilibrium experiments on 0.03 % Hp 2-2. The experimental conditions are the same as in fig. 4.

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in the dissociation. The SH groups do not seem to be involved in the reversible dissociation of haptoglobins, as can be seen from iodacetamide effect, but more probably tyrosine groups take part in this dissociation. It seems easy to understand the dissociation of Hp 1-1 because, according to Shim and Bearn (1965), this protein is constituted of two α and two β chains, the protomer (Monod and al 1965) being one α and one β chain. For Hp 2-2 the present results indicate a subunit of the same molecular weight as for Hp 1-1.

However, according to Connell and al (1966) the molecular weight of an α chain of Hp 2-2 (2α) would be twice that of Hp 1-1 (1α). A greater tendency to aggregation for 2α chains noted by Connell in different conditions may explain this discrepancy.

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