KINETICS OF THE POLYMERIZATION OF HEMOGLOBIN S: STUDIES BELOW NORMAL ERYTHROCYTE HEMOGLOBIN CONCENTRATION

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SUMMARY

The kinetics of polymerization of deoxyhemoglobin S have been studied by measuring transverse water proton relaxation times (T_2) in hemoglobin solutions. As seen by other techniques, the kinetic profile consists of a delay time followed by a decrease in T_2 during polymerization. The length of the delay time can be decreased and the rate of change of T_2 can be increased by increasing the concentration of hemoglobin S or non-gelling hemoglobin or ovalbumin. At a total protein concentration of about 210 mg/ml the kinetic profiles in all three cases are indistinguishable suggesting that a non-specific protein-protein interaction may be involved in the kinetics of polymerization. In addition, it is suggested that no polymer formation occurs during the delay period.

The kinetics of the sol-gel transition of deoxyhemoglobin S have recently been studied by a variety of different methods. Widely different techniques such as viscosity (1,2), calorimetry (3), birefringence (3), turbidity (4) and nuclear magnetic resonance water line width measurements (5) have all shown that following elevation of the temperature of a solution of deoxyhemoglobin S there is a delay period prior to the onset of polymerization. Gel formation follows the delay period and is a rapid process. The length of the delay time has been found to be dependent on the hemoglobin concentration and the temperature to which the system is jumped.

Measurement of transverse water proton relaxation times (T_2) has been used to monitor the polymerization of deoxyhemoglobin S in solutions and in intact erythrocytes (6-9). These studies have led to the hypothesis that the observed decrease in T_2 is due to an increase in the rotational correlation time of a small fraction water irrotationally bound to the hemoglobin molecules as polymerization proceeds (7) and that the T_2 measurements are sen-

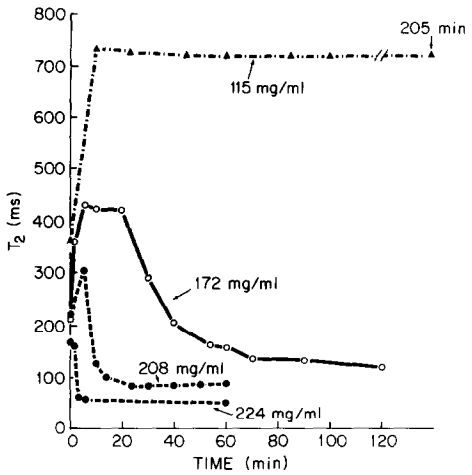

Fig. 1. Transverse water proton relaxation times (T_2) as a function of time at different deoxyhemoglobin S concentrations. The time zero T_2 values were measured at 4°C and all other T_2 values were measured at 37°C. The experiments were carried out as described in the text and the pH in all samples was 7.0.

sitive to a range of correlation times from a single hemoglobin molecule to linear polymers of seven hemoglobin molecules (9). Since this technique is sensitive to the early stages of the polymerization process, it has now been applied to the study of the kinetic properties of polymerization.

MATERIALS AND METHODS

The spin-echo measurements were carried out as previously described (6-9). All samples of hemoglobins A, S and F used were electrophoretically homogeneous. Samples were equilibrated with 0.25 M potassium phosphate buffer, pH 7.4, by dialysis and concentrated by ultrafiltration. Deoxygenation was carried out at 4°C by addition of 0.5 M sodium dithionite dissolved in the same buffer to a final concentration of 46 mM. The buffer was deoxygenated by bubbling with 95% N_2-5% CO_2 before the addition of dithionite. Under the conditions described here, the final pH of all samples was found to be 7.0. Hemoglobin concentrations were determined by measuring the absorbance at 419 nm after reduction with sodium dithionite and bubbling with carbon monoxide (ε=191 $mM^{-1}cm^{-1}$) (10). Mixtures of hemoglobins were prepared from stock solutions of the homogeneous hemoglobins. These samples were then either concentrated by ultrafiltration or diluted with 0.25 M phosphate buffer pH 7.4 to achieve the desired concentrations.

The kinetics of deoxyhemoglobin S polymerization were measured in the following manner. Samples were equilibrated at 4°C for one hour and the T_2 measurement was taken at 4°C. The temperature of the sample was rapidly changed to 37°C and T_2 measurements were taken at time intervals until no further change was observed. Temperature equilibration of the sample from 4°C to 37°C takes no longer than one minute.

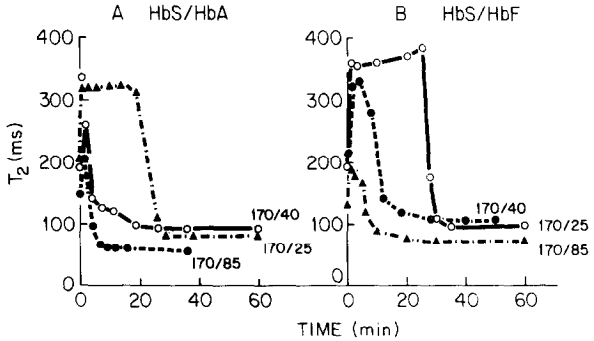

Fig. 2. Transverse water proton relaxation times (T_2) as a function of time in solutions of deoxyhemoglobin S and either deoxyhemoglobin A or F. The experiments were carried out in the same way as those in Figure 1. In panel A are shown the results of mixtures of hemoglobin S and hemoglobin A. In all cases, the final concentration of hemoglobin S was 170 mg/ml. The final hemoglobin A concentration was varied as follows: 25 mg/ml (▲); 40 mg/ml (O); 85 mg/ml (●). In panel B are shown the results of mixtures of hemoglobin S and hemoglobin F. In all cases, the final concentration of hemoglobin S was 170 mg/ml. The final hemoglobin F concentration was varied as follows: 25 mg/ml (O); 40 mg/ml (●); 85 mg/ml (▲).

RESULTS AND DISCUSSION

In Figure 1, the change in T_2 as a function of time is shown at four different concentrations of hemoglobin S. At 115 mg/ml, T_2 increases as the temperature is jumped from 4°C to 37°C, but no further change is observed. The increase is due to a decrease in the correlation time of the irrotationally bound water as the temperature is increased. However, no polymerization occurs at this protein concentration. At 172 mg/ml, T_2 increases as the temperature is jumped from 4°C to 37°C, then for a period of about 20 minutes there is no change in T_2, then T_2 decreases as polymerization of the deoxyhemoglobin S proceeds. At 208 mg/ml, T_2 initially increases then rapidly decreases as polymerization takes place. Above 220 mg/ml, there is no measurable increase in T_2 initially, only the rapid decrease.

To study the effect of non-gelling hemoglobins on the polymerization of deoxyhemoglobin S, the concentration of 170 mg/ml of hemoglobin S was held

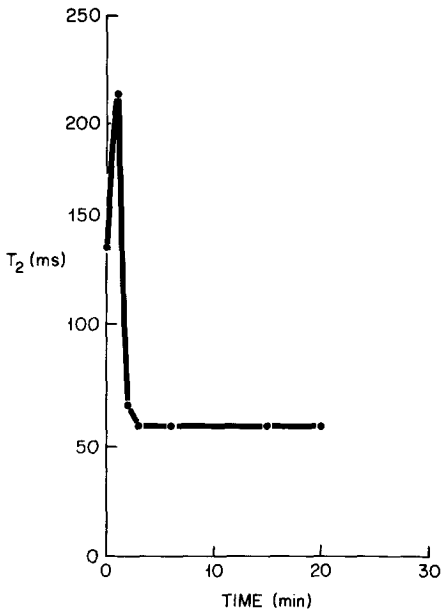

Fig. 3. Transverse water proton relaxation times (T_2) as a function of time in a solution containing 170 mg/ml hemoglobin S and 40 mg/ml ovalbumin. The experiment was carried out in the same way as those in Figure 1.

constant and different concentrations of the non-gelling hemoglobin were added. The results of these studies with hemoglobin A are shown in Figure 2-A, and with hemoglobin F in Figure 2-B. At all concentrations of non-gelling hemoglobin, the rate of the overall polymerization process is accelerated. Upon addition of 25 mg/ml of either hemoglobin A or F to 170 mg/ml of hemoglobin S prior to the temperature jump, the delay time is about 20 minutes, the same as observed with 172 mg/ml hemoglobin S. However, as seen in Figure 1, when no non-gelling hemoglobin is present, the total change in T_2 occurs over about a 40 minute time period, whereas in Fig. 2, this process takes only about 10 minutes. Therefore, at low concentrations, non-gelling hemoglobins do not seem to alter the delay time, but they do accelerate the rate of the change in T_2. Higher concentrations of non-gelling hemoglobins decrease the length of the delay time and further increase the rate of change of T_2 dur-

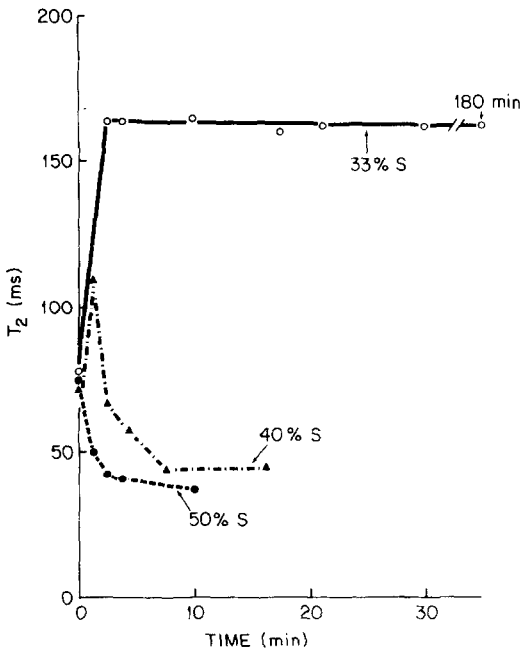

Fig. 4. Transverse water proton relaxation times (T_2) as a function of time in hemoglobin solutions containing varying amounts of hemoglobin S. Each solution studied contained a total hemoglobin A + hemoglobin S concentration of 340 mg/ml. However, the percentage of hemoglobin S was different in each solution, as indicated in the figure. The experiments were carried out in the same way as those in Figure 1.

ing polymerization. Higher concentrations of hemoglobin S have the same effect. Note that the profiles of 208 mg/ml hemoglobin S (Fig. 1) and 170 mg/ml hemoglobin S plus 40 mg/ml hemoglobin A or F (total concentration of 210 mg/ml, see Fig. 2) are the same. In Fig. 3 the results of an experiment using 170 mg/ml hemoglobin S and 40 mg/ml ovalbumin are shown. In this case the kinetic profile is similar to those seen in Fig. 1 or Fig. 2 for a total hemoglobin concentration of 210 mg/ml. Therefore the decrease in the length of the delay time and the increase in the rate of change of T_2 which results from an increase in hemoglobin S concentration or an increase in non-gelling hemoglobin concentration are primarily due to a non-specific protein concentration effect.

In Fig. 4 are shown the results of kinetic experiments carried out at a

constant total hemoglobin concentration of 340 mg/ml (approximately that found in erythrocytes). The percentage of hemoglobin S has been varied, and the remainder of the hemoglobin is hemoglobin A. As can be seen, a critical level of hemoglobin S must be present for polymerization to take place (somewhere above 33%). It will be noted that the hemoglobin S at 33% in this experiment is 112 mg/ml and no polymerization occurs just as with 115 mg/ml hemoglobin S in Fig. 1. The additional protein concentration (hemoglobin A) in this case has no influence because the hemoglobin S concentration is below a critical level. However, once the critical level of hemoglobin S is exceeded, (somewhere below 40% or 136 mg/ml) other proteins such as hemoglobin S or non-gelling hemoglobin (hemoglobin A or F) or ovalbumin all accelerate the rate of the polymerization by a non-specific process. At a total protein concentration of 210 mg/ml (well below erythrocyte hemoglobin concentrations) the results in all three cases are indistinguishable by this method.

Eaton and his colleagues (11) have suggested that the kinetics of polymerization may be important clinically and that lengthening the delay time might be of therapeutic value. It seems clear from the results presented above that the search for ways of lengthening the delay need not be limited to hemoglobin specific agents but rather should be considered in light of protein interactions in general.

Also, since the total change in T_2 observed using this 24.3 MHz pulsed NMR spectrometer can be explained by the correlation times expected upon polymerization of deoxyhemoglobin S molecules up to a linear polymer of seven (9), it must be suggested that no significant polymerization occurs for about 20 min. following a temperature jump from 4°C to 37°C at 172 mg/ml of deoxyhemoglobin S. The delay time is not characterized by a significant amount of stable linear small polymer formation. Also the data in Fig. 2 suggest that the delay time and the rate of change of T_2 (polymerization) have different concentration dependencies. Although differences between the effect of hemoglobins A and F are well known in equilibrium studies, no obvious differences are observed in these kinetic studies.

In summary, the kinetic profile of deoxyhemoglobin S polymerization obtained from T_2 measurements is similar to that obtained by other methods. It is found, however, that increasing concentrations of hemoglobin S, hemoglobin A or F, or ovalbumin all have the same effect of decreasing the length of the delay time and increasing the rate of polymerization, presumably due to a non-specific protein-protein interaction. It is also suggested that no significant small polymer formation occurs during the delay period prior to the onset of polymerization.

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