Sickling of erythrocytes occurs when deoxygenation and the development of suitable therapy has been slow. Unlike Fe(II)HbS, the first heme-substituted hemoglobin shown to mimic the polymerization of deoxyHbS. Unlike Fe(II)HbS, Ni(II)HbS does not bind oxygen and therefore polymerizes under aerobic conditions. While the polymer solubility coefficient \( C_{wa} \) for Ni(II)HbS is elevated about 2 g/100 ml compared with that for deoxy Fe(II)HbS, hemoglobin concentration in the polymer phase is the same. Electron micrographs of thin sections of embedded Ni(II)HbS reveal 20-nm-diameter fibers indistinguishable from those seen with deoxygenated native HbS. Nickel(II)HbS can be used in studies on the sickling process and on antisickling agents that could not previously be done or were difficult to execute because of the need for an anaerobic environment.

Sickle cell disease (SCD) has been the focus of intense research. However, the detailed molecular basis of the sickling process is not yet fully understood and the development of suitable therapy has been slow. Sickling of erythrocytes occurs when deoxygenation causes an allosteric transition of protein conformation \((R \rightarrow T)\), which brings the mutation site, \( \beta 6 \text{Glu} \rightarrow \text{Val} \), of one hemoglobin S (HbS) molecule in register with a receptor site on the \( \beta \) chain of another HbS molecule. This triggers a nucleation process with subsequent fiber formation, which ultimately distorts the red cell membrane.

It has been demonstrated by Alston et al.\(^2\) that normal hemoglobin (HbA) reconstituted with Ni(II)-protoporphyrin IX exists in a deoxyconformational state, does not bind \( O_2 \) or CO, is not oxidized in the presence of air, and is not photolabile. These properties give the bright pink Ni(II)HbA preparation, and presumably by analogy the reconstituted hemoglobin S analogue, the unusual ability of maintaining a deoxy T-like state in aerobic conditions. Recently, Ni(II)HbS was proposed as a possible model compound for studying the polymerization process and for designing drugs to inhibit gelation,\(^3\) but its ability to form a gel in ultracentrifugation studies and to form fibers demonstrable by electron microscopy (EM) had yet to be established. We report here that, like native HbS, Ni(II)HbS forms a gel in anaerobic environments. Furthermore, unlike native HbS, it gels in aerobic environments as well. It can therefore be used to investigate the antisickling properties of drugs that cannot be studied in the presence of dithionite because they are reduced by this reagent. It can also be used in mixture experiments done at atmospheric \( P_{O_2} \), in which Ni(II)HbS is held in the deoxy T state while other hemoglobins are in the oxygenated R state. While the polymer solubility coefficient \( C_{wa} \) for Ni(II)HbS is somewhat higher than for native HbS, hemoglobin concentration in the pellet is comparable. Electron microscopic studies show that the fiber structures of Ni(II)HbS are indistinguishable from those of native deoxyHbS.

MATERIALS AND METHODS

Hemoglobin S was purified on DEAE-cellulose columns using 0.05 mol/L TRIS-HCl, pH 8.1.\(^4\) Nickel(II)HbS was prepared according to the technique of Alston et al.\(^2\) The \( C_{wa} \) data were obtained by ultracentrifugation assay\(^5\) under the conditions described previously\(^7\) in which dithionite is used to achieve and maintain an anaerobic environment. For aerobic experiments, neither nitrogen flushing nor dithionite was used. The nickel and iron hemoglobin were handled identically except that in choosing initial pHs, consideration had to be given to the smaller \( P_{O_2} \) content of Ni(II)Hbs solutions (which would reduce dithionite related \( pH \) changes) and to the lack of Bohr effect in Ni(II)HbS. Therefore, the starting \( pH \) for the dialysis buffer in the concentration step had to be individualized to give a final \( pH \) of 6.80 to 6.90 for the gel supernatants in all cases. The pellet concentration of the centrifuged gel was measured as described by Benesch et al.\(^6\) except that for the Ni(II)HbS, the pellet was dissolved in 0.1 mol/L potassium phosphate buffer, \( pH \) 7.3. The previously reported\(^2\) extinction coefficient at 398 nm was used to calculate the concentration of Ni(II)HbS.

RESULTS AND DISCUSSION

A comparison of \( C_{wa} \) for Ni(II)HbS and native HbS controls is seen in Table 1. As expected, native HbS did not form a gel under aerobic conditions. The \( C_{wa} \) for deoxy HbS in the presence of dithionite is in excellent agreement with previously reported data under identical conditions of temperature, \( pH \), and ionic strength,
NICKEL (II) HEMOGLOBIN S POLYMERIZES

Table 1. Aerobic and Anaerobic Gelation and Ultracentrifugation Performed at 25 °C in 0.1 mol/L Potassium Phosphate Buffer

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Added Salt</th>
<th>Hemoglobin Concentration g/100 mL</th>
<th>Supernatant (Cₕ)</th>
<th>Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native HbS</td>
<td>None</td>
<td>30 mmol/L Na₂S₂O₄</td>
<td>17.0</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>(Aerobic)</td>
<td>17.3</td>
<td>50.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 mmol/L Na₂S₂O₄</td>
<td>17.1</td>
<td>51.9</td>
</tr>
<tr>
<td>Ni (II) HbS</td>
<td>None</td>
<td>30 mmol/L NaCl</td>
<td>19.1</td>
<td>49.6</td>
</tr>
<tr>
<td></td>
<td>(Aerobic)</td>
<td>19.3</td>
<td>48.1</td>
<td></td>
</tr>
<tr>
<td>Ni (II) HbS</td>
<td>100 mmol/L NaCl</td>
<td>19.3</td>
<td>48.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 mmol/L Na₂S₂O₄</td>
<td>19.7</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>(Anaerobic)</td>
<td>19.6</td>
<td>50.4</td>
<td></td>
</tr>
</tbody>
</table>

Since dithionite alters pH, to ensure comparable final pHs, various starting pHs for the buffer in the ultrafiltration concentration step were tried. The data reported are for experiments that gave a final pH of 6.8 to 6.9 for the gel supernatant. Initial hemoglobin concentration before gelation was 22 to 25 g/100 mL in all cases.

which in multiple determinations give a value of 17.4 ± 0.3 g/100 mL. Nickle(II)HbS was studied with dithionite (anaerobic) and without dithionite (aerobic—with and without added NaCl in an attempt to substitute for the ionic strength contribution of dithionite). In all cases a gel formed and the Cₛ for Ni(II)HbS was slightly higher than that for native deoxy HbS. The largest difference occurred when both the Ni(II)HbS and native HbS were studied in the presence of dithionite and thus were under identical conditions. Since the proportions of various dithionite breakdown products in a solution are ill defined, it is hard to match the ionic strength of samples with and without dithionite, so two concentrations of NaCl were used. The somewhat elevated Cₛ for Ni(II)HbS supports previous data that indicate that the solubility of Ni(II)HbS in high ionic strength phosphate is increased compared with that of deoxy HbS.

The pellet Hb concentration cannot be determined as accurately as the Cₛ, but the data here indicate no statistically significant difference between the groups of samples (Table 1). In summary, the ultracentrifugation data are consistent with nickel substitution producing a rather small alteration of contact sites between tetramers, but they provide no evidence for alteration in fiber structure or packing.

Using the electron microscope, we found that the fibers formed by Ni(II)HbS in aerobic environments are indistinguishable from the polymerized structures that can be demonstrated for native HbS only after deoxygenation. Electron micrographs of Ni(II)HbS (Fig 1a) show that the fibers have a typical appearance from a longitudinal view. The long parallel striaations are consistent with all deoxy HbS derivatives known to polymerize. In Figure 1b, EM results obtained from a cross-sectional cut of embedded Ni(II)HbS yielded a fiber of normal size (diameter of ~20 nm) with various detailed patterns (eg, spirals, bull’s-eyes) reported previously for native HbS. It has been shown that these patterns can be interpreted as cross sections of a 14-filament structure viewed end-on or nearly end-on.

In conclusion, Ni(II)HbS polymerizes under aerobic conditions yielding fibers that are indistinguishable in structure and packing from those of native deoxy HbS. This metal substituted hemoglobin can therefore serve as a model for studying HbS polymerization in an aerobic environment. This allows the design and implementation of studies on the sickling process and on potential antisickling agents that are difficult or impossible to perform in the presence of dithionite or under strictly anaerobic conditions.

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The polymerization of nickel (II) hemoglobin S under aerobic conditions

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