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Dipeptides as Inhibitors of the Gelation of Sickle Hemoglobin

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SUMMARY

To examine in detail a class of peptides that inhibit the polymerization of deoxyhemoglobin S, we assayed the L-amino acids and 22 dipeptides for their effect on deoxyhemoglobin S solubility. Of the amino acids, the aromatics (phenylalanine, tyrosine, and tryptophan) significantly increased deoxyhemoglobin S solubility, as did high concentrations of arginine. Combinations of the hydrophobic (specifically the aromatic) amino acids with a hydrophilic amino acid, such as arginine or lysine, resulted in dipeptides which were much more soluble than the hydrophobic or aromatic amino acid alone, and also inhibited polymerization. Furthermore, samples of deoxyhemoglobin S at 26 to 27 g/dl containing some of these dipeptides such as Arg-Trp, Arg-Phe, and Lys-Trp in excess of 50 to 100 mM did not polymerize, indicating a 1.4- to 1.6-fold increase in deoxyhemoglobin S solubility. The enhancement of polymerization, i.e., decrease in deoxyhemoglobin S solubility, observed by the addition of aspartic acid, glycine, or lysine was observed or was reduced in the dipeptides containing these hydrophilic amino acids combined with hydrophobic amino acids (valine, leucine, isoleucine, or the aromatic amino acids). The effects of these dipeptides on deoxyhemoglobin S solubility were mostly linear with concentration. However, the changes in deoxyhemoglobin S solubility by addition of a dipeptide was not simply the sum of the effects observed with the individual amino acids as exemplified by the differential effect of reversing the dipeptide sequence (e.g., Arg-Phe and Phe-Arg, or Arg-Tyr and Tyr-Arg). These data provide further evidence as to the stereospecific nature of this class of noncovalent inhibitors of deoxyhemoglobin S polymerization.

INTRODUCTION

The substitution of valine for glutamic acid in the β6-position of sickle hemoglobin results in a reduction of the solubility of hemoglobin S solution to 17 g/dl upon deoxygenation under physiological conditions (1). In SS erythrocytes (cells from individuals homozygous for the sickle cell gene), in which the mean intracellular hemoglobin concentration is about 34 g/dl, the low solubility of deoxyhemoglobin S causes hemoglobin S gelation or polymer formation upon deoxygenation (2). In order to examine the intermolecular contacts of the hemoglobin S polymer, Wishner *et al.* (3) solved at 5-Å resolution a crystal structure of deoxyhemoglobin S and identified the relevant feature related to hemoglobin S gelation as paired "strands" of deoxyhemoglobin S tetramers. Several studies using electron microscopy and X-ray fiber diffraction have utilized this information to predict the fiber structure of the deoxyhemoglobin S gel or polymer and the detailed orientation of the hemoglobin S tetramers (4–6).

We have used the structural information derived from X-ray crystallography and electron microscopy in an effort to design noncovalent inhibitors of deoxyhemoglobin S polymer formation. Specifically, we are examining the ability of small peptides to interfere with the intermolecular binding between two tetramers. These studies began with our discovery that aromatic amino acids could inhibit deoxyhemoglobin S polymer formation (7, 8). However, an aromatic group alone was not sufficient to increase maximally the deoxyhemoglobin S solubility. Ross and Subramanian (9) and Behe and Englander (10) examined a number of aromatic compounds, while Poillon and Kim (11) and our laboratory (12) continued to study a number of derivatives of amino acids. As proposed by Ross and Subramanian (9), this class of noncovalent inhibitors may be acting in a stereospecific manner by interfering with the intermolecular contact in the regions of β73(Asp), β85(Phe), and β88(Leu) on one hemoglobin tetramer and in the region of β4(Thr) and β6(Val) on an adjacent tetramer.

Kubota and Yang (13) initially studied peptides which

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might mimic the intermolecular binding site in the region of β6(Val) as potential inhibitors of gelation. However, a number of peptides from the amino-terminal region of the β-chain of hemoglobin S (β1–12) that we tested enhanced gelation (8), possibly as a result of an excluded volume (nonideality) effect of these peptides in concentrated hemoglobin solutions (the activity coefficient of hemoglobin at 34 g/dl is about 50) (2). Gorecki *et al.* (14) examined a number of small peptides and found that some of those containing L-Phe or L-Trp could indeed inhibit gelation. Most recently, Franklin *et al.* (15) reported on the ability of L-Lys-L-Phe to inhibit gelation. To define further this class of noncovalent inhibitors of gelation, we now report on a systematic study of 22 dipeptides. We examine the possibility of increasing the effectiveness of these inhibitors by combining a hydrophobic amino acid with a hydrophilic amino acid. The increased solubility of some of these dipeptides relative to the aromatic amino acid alone permits a more detailed examination of the inhibition of gelation by these compounds. These studies also enable us to examine the stereospecific nature of binding more explicitly.

MATERIALS AND METHODS

Chromatographically pure hemoglobin S was prepared from SS erythrocytes, washed three times in saline, and lysed in 10 times the volume of distilled water. The hemolysate was dialyzed against 0.05 M Tris-HCl, pH 8.2, with 1 mM EDTA. Hemoglobin S was isolated using a DEAE-Sephacel (Pharmacia) column with a 0.05 M Tris-HCl, 1 mM EDTA buffer (16). The pH gradient ranged from 8.2 to 7.6. The purity of hemoglobin S was confirmed by isoelectric focusing on a polyacrylamide gel. Hemoglobin S was concentrated by ultrafiltration and vacuum dialysis to 30 g/dl and dialyzed against 0.15 M potassium phosphate buffer, pH 7.3. The amino acids were obtained from Calbiochem (L forms were used in this study). The dipeptide Lys-Leu was obtained from Sigma and the remaining dipeptides were from Vega Biochemicals. All amino acids and dipeptides were the L-isomers. Stock solutions of each of the compounds to be tested were made. The solubility assay developed by Hofrichter *et al.* (17) was used to determine the effect of these dipeptides on deoxyhemoglobin S gelation. Concentrated hemoglobin S, buffer, and the additive to be tested were loaded into 5-mm quartz tubes (Wilmad PQ 701) and kept on ice. Under a nitrogen atmosphere, an ice-cold solution of sodium dithionite (Vine Chemicals, Manchester, England) was added (0.05 M final concentration) to deoxygenate the samples. The tubes were capped and rapidly mixed. The caps were painted with Glyptol (General Electric) to keep them impermeable to oxygen. To mix, the samples were stored under nitrogen and slowly rotated for 16 hr at 5°. The samples were then incubated at 30° to induce gelation. After gelation of the sample, the tubes were spun in the ultracentrifuge at 100,000 × *g* for 3 hr. The total hemoglobin concentration of the sample before gelation and of the supernatant after the ultracentrifugation was determined from the near infrared spectrum using the wavelengths at 1090 and 910 nm. Alternatively, the supernatant concentration was also determined by opening the sample tubes and using a cyanomethemoglobin conversion and optical density at 540 nm. At least two control samples (without additive) were included in each ultracentrifugation spin.

RESULTS

In earlier reports, we examined the effect of amino acids on gelation of deoxyhemoglobin S (7, 8). At 16 mM, only the aromatic amino acids (either L- or D-) significantly increased deoxyhemoglobin S solubility. We have now extended the concentration range tested of some of the amino acids (all L-isomers), tryptophan, phenylalanine, arginine, leucine, isoleucine, valine, lysine, glycine, and aspartic acid, as reference values for understanding the effects of dipeptides on gelation. The data for these amino acids are illustrated in Fig. 1. The solubility for 31 control samples was 17.2 ± 0.5 g/dl.

As reported in our earlier work (8), tryptophan and phenylalanine had the greatest effect in increasing deoxyhemoglobin S solubility (tryptophan at 7 mM increases the solubility by a factor of 1.06 and phenylalanine at 32 mM by 1.13) as shown in Fig. 1. Surprisingly, high concentrations of arginine (100 mM) could increase the solubility by a factor of about 1.15 (about three times the concentration of phenylalanine required for an analogous effect). Leucine, isoleucine, and valine had very small effects on solubility with the solubility the greatest in the presence of leucine and least in the presence of valine (the concentrations assayed were limited by the solubility of the amino acids). In contrast, lysine, glycine, and aspartic acid decreased deoxyhemoglobin S solubility.

The dipeptides we studied were combinations of the relatively hydrophilic amino acids (Arg, Lys, Gly, and Asp) with the hydrophobic amino acids (Trp, Tyr, Phe, Leu, Ile, and Val). The results for the arginyl dipeptides are shown in Fig. 2. For Arg-Trp, Arg-Tyr, and Arg-Phe, the increase in deoxyhemoglobin S solubility appears to be slightly greater than the aromatic amino acid alone (less than 10% greater). However, this effect is not simply the addition of the effect of each amino acid alone. The combination of aromatic amino acids with arginine renders them much more soluble in aqueous solution. Furthermore, samples of Arg-Trp, Arg-Tyr, and Arg-Phe assayed at 50, 75, and 75 mM, respectively (deoxyhemoglobin S at 26 to 27 g/dl), resulted in samples that did not gel even after 24 hr at 30°. Combinations of Ile and

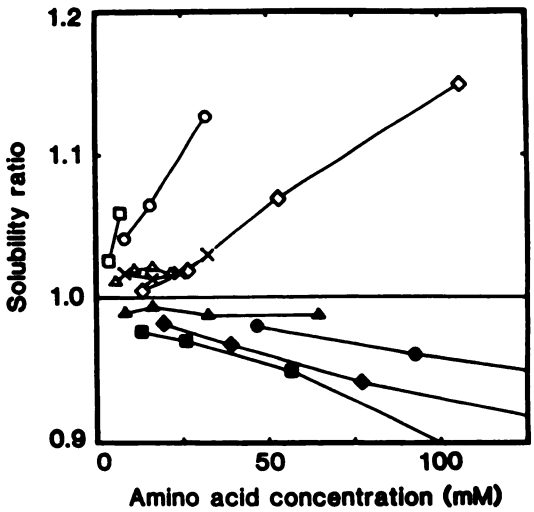

FIG. 1. *The amino acid concentration versus the solubility ratio*
The solubility ratio represents the effect of an additive on deoxyhemoglobin S solubility and is given by the solubility of deoxyhemoglobin S in the presence of the additive divided by the control solubility. A gelation inhibitor increases the solubility and gives values greater than 1. A gelation enhancer decreases the solubility and gives values less than 1. □, Trp; ○, Phe; ◇, Arg; △, Leu; ×, Ile; ▲, Val; ●, Lys; ◆, Gly; ■, Asp.

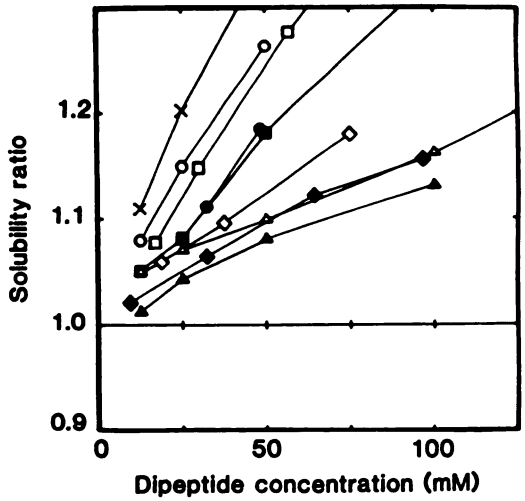

FIG. 2. *Arg dipeptide concentration versus the solubility ratio (see Fig. 1)*

×, Arg-Trp; ○, Arg-Tyr; □, Arg-Phe; ○, Tyr-Arg; ■, Phe-Arg; ◇, Arg-Leu; ◆, Leu-Arg; △, Arg-Ile; ▲, Arg-Val.

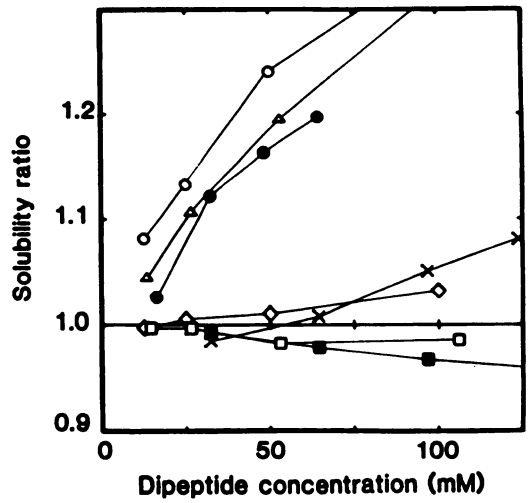

FIG. 3. *Lys dipeptide concentration versus the solubility ratio (see Fig. 1)*

○, Lys-Tyr; △, Lys-Phe; ○, Tyr-Lys; ×, Lys-Leu; ◇, Lys-Ile; □, Lys-Val; ■, Val-Lys.

Val with arginine increased the effect on solubility compared to that of arginine alone. The order of the modifying effect (Arg-Leu > Arg-Ile > Arg-Val) is the same as the order of the solubility of deoxyhemoglobin S with only hydrophobic amino acids present (Leu > Ile > Val).

The results for the lysyl dipeptides are shown in Fig. 3. In contrast to arginine, lysine decreases deoxyhemoglobin S solubility and the lysine dipeptides were less effective than the arginine dipeptides in increasing deoxyhemoglobin S solubility. Lys-Tyr and Lys-Phe increased deoxyhemoglobin S solubility to the same extent as tyrosine and phenylalanine alone. The hydrophilic nature of lysine enabled us to examine Lys-Leu, Lys-Ile, and Lys-Val at concentrations much higher than Leu, Ile, or Val alone. At low concentrations, Lys-Leu, Lys-Ile, and Lys-Val had no effect or had a much smaller effect on reducing deoxyhemoglobin S solubility as compared to lysine alone. At high concentrations (>100 mM), Lys-Leu (and possibly Lys-Ile) increased deoxyhemoglobin S solubility. This result may be attributed to the presence of Leu alone, but is difficult to determine due to the limited solubility of free leucine.

The results for the glycyl dipeptides and aspartyl dipeptides are illustrated in Fig. 4. Gly-Phe and Asp-Phe increased deoxyhemoglobin S solubility to the same extent as the effect of phenylalanine alone and Lys-Phe. Asp-Leu and Asp-Val exhibited limited solubility and little or no effects at low concentrations. At the maximal concentrations tested, Asp-Leu (40 mM) increased deoxyhemoglobin S solubility by a factor of 1.07. Gly-Val and Gly-Ile were more soluble than valine or isoleucine alone. Both dipeptides reduced the effect of glycine alone on decreasing deoxyhemoglobin S solubility. In fact, Gly-Ile at 120 mM increased the solubility by a factor of 1.13. This may reflect the effect of isoleucine alone, but again this is difficult to determine because of the limited solubility of free isoleucine.

The effectiveness of these dipeptides and amino acids appears generally to be linear with concentration. In order to facilitate comparison among the various dipeptides, we used a linear regression analysis of the data [(solubility ratio) = 1 + slope × additive concentration] and designated the slope as a relative index of effectiveness (Table 1). A positive, negative, or zero slope is obtained for compounds which increase, decrease, or have no effect on deoxyhemoglobin S solubility, respectively. By this analysis (Table 1), tryptophan (m = 8.5/M) is 2-fold more effective than phenylalanine (m = 4.1/M) while arginine (m = 1.3/M), leucine (m = 1.1/M), and isoleucine (m = 0.9/M) are much less effective by a factor of 4 or more. The negative slopes for lysine, glycine, and aspartic acid explicitly demonstrate an increasing tendency for these amino acids to enhance gelation.

The values of the slopes for the aromatic dipeptides are similar to those observed for the single aromatic amino acids (tryptophan and phenylalanine) and are between 3/M and 8/M. The slopes for the nonaromatic dipeptides are between −0.3/M and 2.5/M, less than the

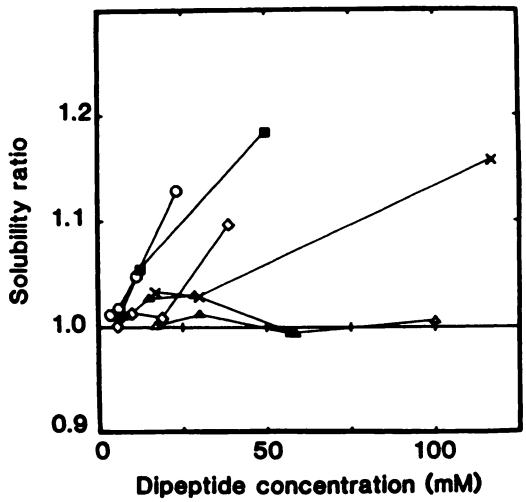

FIG. 4. *Gly or Asp dipeptide concentration versus the solubility ratio (see Fig. 1)*

○, Gly-Phe; ■, Asp-Phe; ◇, Asp-Leu; ×, Gly-Ile; ▲, Asp-Val; △, Gly-Val.

TABLE 1

Slopes of effect on deoxyhemoglobin S solubility

Amino acid	$m(1/M)^a$
Trp	8.5
Phe	4.1
Arg	1.3
Leu	1.1
Ile	0.9
Val	−0.2
Lys	−0.4
Gly	−0.7
Asp	−1.0
Aromatic dipeptides	
Arg-Trp	7.3
Arg-Tyr	5.4
Tyr-Arg[b]	3.7
Arg-Phe	4.8
Phe-Arg[b]	3.4
Lys-Tyr	5.0
Tyr-Lys[b]	3.2
Lys-Phe	3.7
Gly-Phe	5.1
Asp-Phe	3.7
Nonaromatic dipeptide	
Arg-Leu	2.5
Leu-Arg[b]	1.8
Arg-Val	1.4
Lys-Leu	0.5
Lys-Ile	0.3
Lys-Val	−0.2
Val-Lys[b]	−0.3
Gly-Ile	1.3
Gly-Val	0.0
Asp-Leu	2.0
Asp-Val	NC[c]

[a] The slope m is determined by fitting the data to the form (solubility ratio = $1 + m \times$ additive molar concentration) and is in units of (1/M).

[b] The hydrophobic-hydrophilic dipeptide (reversing the amino acid sequence) is indicated in italics directly below the original hydrophilic-hydrophobic sequence.

[c] Asp-Val deviated the most from the linear behavior described in footnote *a*, and the slope value for this dipeptide was not calculated (NC).

values for the aromatic dipeptides but greater than the values observed for lysine, glycine, or aspartic acid alone. (That is, these dipeptides have a reduced potential to enhance gelation.)

DISCUSSION

The aromatic amino acids, particularly Phe and Trp, are among the most effective noncovalent inhibitors of deoxyhemoglobin S gelation (8). The other hydrophobic amino acids, Leu and Ile but not Val, also exhibited a small inhibitory effect on polymerization. Unlike the other hydrophilic amino acids (Asp, Lys, and Glu) which enhanced gelation, Arg also inhibited gelation. The manner in which Arg interacts with the deoxyhemoglobin S molecule to inhibit gelation is unclear but it is unlikely that the hydrophobic interaction proposed for Phe and Trp would be relevant for such a hydrophilic amino acid. The enhancement of gelation by Lys, Asp, Gly, several other amino acids, and oligopeptides that we previously reported may be a solvent-mediated or salting out phenomenon (18). Enhancement of gelation by Lys, Asp, and Gly appears to be minimized or eliminated when these amino acids are combined with Leu or Ile. Furthermore, the large inhibitory effect on gelation of Phe alone appears unaffected when combined with Lys, Asp, or Gly. In general, the mechanism responsible for the decrease in deoxyhemoglobin S solubility by Lys, Asp, or Gly is less evident when these amino acids are incorporated into dipeptides.

The combination of a hydrophilic amino acid (Arg, Lys, or Asp) with a hydrophobic amino acid (Phe, Trp, Tyr, Leu, or Ile) results in a dipeptide with greater solubility than the hydrophobic amino acid alone. The results for the dipeptides containing Tyr are particularly interesting in view of the limited solubility of Tyr alone. As indicated in Figs. 1–3 and Table 1, dipeptides containing Trp or Phe inhibited gelation with Arg-Trp > Arg-Tyr > Arg-Phe and Lys-Tyr > Lys-Phe in order of effectiveness. This indicates that the order of the effectiveness of the hydrophobic amino acids alone is most likely Trp > Tyr > Phe. The dipeptides containing Arg were more effective in inhibiting gelation than the Lys or Asp dipeptides relating to the inhibitory potential of Arg alone. However, the increase in deoxyhemoglobin S solubility of Arg-Phe and Arg-Trp is less than the sum of the relative increase of each amino acid alone, indicating that the inhibition of gelation by the dipeptides is not simply additive. Gorecki *et al.* (14) examined several peptides and concluded that the hydrophobicity of the side chains is the predominant factor in determining the inhibitor potential of a peptide. While this would explain much of our results with the Phe, Trp, Tyr, Leu, and Ile dipeptides, the unusual results obtained with Arg would indicate that, at least under certain circumstances, charge-related interactions appear to be involved.

The stereospecificity of the interaction between these dipeptides and deoxyhemoglobin S was examined using the reverse sequence of several dipeptides. We found that the sequence of hydrophilic-hydrophobic dipeptides was more effective in inhibiting gelation than the respective reverse sequence. Specifically, the inhibitory potentials of the dipeptides were Arg-Tyr > Phe-Arg and Arg-Leu > Leu-Arg (Fig. 2) and Lys-Tyr > Tyr-Arg (Fig. 3). [We also observed that both Val-Lys and Lys-Val had little effect or decreased deoxyhemoglobin S solubility (Fig. 3).] These results support the hypothesis that the mechanism of action of these noncovalent inhibitors of gelation is stereospecific in nature. Recently, Franklin *et al.* (15) reported on the inhibition of gelation by Lys-Phe. The dramatic increase in solubility reported for Lys-Phe in comparison to Phe or Phe-Lys appears to be consistent with results presented here that the effectiveness of Arg-Tyr, Arg-Phe, and Lys-Tyr is greater than the reverse sequence.

While Phe and Lys-Phe have been demonstrated to be taken up by the erythrocyte (15), this process is slow and reversible. Lipid vesicles loaded with Phe have been used to further increase the intracellular concentration of Phe by fusing such vesicles with the erythrocyte (19, 20). However, the leaking out of Phe from the erythrocyte remains a difficult problem. Gorecki *et al.* (21) have examined the effect of chemical modification of Phe to improve erythrocyte uptake. Although phenylalanine-benzyl ester is readily incorporated into the erythrocyte, probably binding to the membrane, it does not have any

effect on deoxyhemoglobin S solubility and causes hemolysis. Several techniques have been employed to determine specific binding sites. Abraham *et al.* (22) have been examining the binding using X-ray crystallographic techniques (however, no single binding site for either phenylalanine, Lys-Tyr, or Arg-Tyr has yet been determined in crystals of hemoglobin grown in the presence of these inhibitors). Spin label-induced nuclear magnetic relaxation studies of phenylalanine and hemoglobin indicate phenylalanine binds weakly, and possibly in the region around β85(Phe), β88(Leu), β91(Leu), or a segment of the β-heme porphyrin ring (23). Proton nuclear magnetic resonance spectroscopy data further suggest that phenylalanine and tryptophan have a binding site in the region of β6(Val) on hemoglobin S (which is not present on hemoglobin A) and in the region of the heme pockets of the α- and β-chains on both hemoglobins A and S (24).

As demonstrated here, dipeptides represent an effective class of noncovalent inhibitors of deoxyhemoglobin S polymerization. Addition of Arg-Trp, Arg-Phe, and Lys-Trp in excess of 50 to 100 mM can increase deoxyhemoglobin S solubility by 1.4- to 1.6-fold, equivalent to the increase in deoxyhemoglobin solubility observed for the clinically benign sickle trait condition (25, 26). Underscoring the stereospecific nature of this interaction is the fact that reversing the order of the amino acids in some of these dipeptides can reduce its inhibitory potential. However, the therapeutic utility of these compounds is at present limited by the high concentrations required and the lack of specific and irreversible uptake into the red cell.

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REFERENCES

1. Dean, J., and A. N. Schechter. Sickle-cell anemia: molecular and cellular bases of therapeutic approaches. *N. Engl. J. Med.* **299**:752–763, 804–811, 863–870 (1978).
2. Noguchi, C. T., and A. N. Schechter. The intracellular polymerization of sickle hemoglobin and its relevance to sickle cell disease. *Blood* **58**:1057–1068 (1981).
3. Wishner, B. C., K. B. Ward, E. E. Lattman, and W. E. Love. Crystal structure of sickle-cell deoxyhemoglobin at 5 Å resolution. *J. Mol. Biol.* **98**:179–194 (1975).
4. Edelstein, S. J. Molecular topology in crystals and fibers of hemoglobin S. *J. Mol. Biol.* **150**:557–575 (1981).
5. Vassar, R. J., M. J. Potel, and R. Josephs. Studies of the fiber to crystal transition of sickle cell hemoglobin in acidic polyethylene glycol. *J. Mol. Biol.* **157**:395–412 (1982).
6. Rosen, L. S., and B. Magdoff-Fairchild. Molecular packing in a second monoclinic crystal of deoxygenated sickle hemoglobin. *J. Mol. Biol.* **157**:181–189 (1982).
7. Noguchi, C. T., and A. N. Schechter. Effects of amino acids on gelation kinetics and solubility of sickle hemoglobin. *Biochem. Biophys. Res. Commun.* **74**:637–642 (1977).
8. Noguchi, C. T., and A. N. Schechter. The inhibition of sickle hemoglobin gelation by amino acids and related compounds. *Biochemistry* **17**:5455–5459 (1978).
9. Ross, P. D., and S. Subramanian. Inhibition of sickle cell hemoglobin gelation by some aromatic compounds. *Biochem. Biophys. Res. Commun.* **77**:1217–1223 (1977).
10. Behe, M. J., and S. W. Englander. Quantitative assessment of the noncovalent inhibition of sickle hemoglobin gelation by phenyl derivatives and other known agents. *Biochemistry* **18**:4196–4201 (1979).
11. Poillon, W. N., and B. C. Kim. Noncovalent inhibitors of sickle hemoglobin gelation: effects of aryl-substituted alanines. *Biochemistry* **21**:1400–1406 (1982).
12. Noguchi, C. T., S. Ackerman, J. DiMaio, P. W. Schiller, and A. N. Schechter. The effect of phenylalanine derivatives on the solubility of deoxyhemoglobin S: a model class of gelation inhibitors. *Mol. Pharmacol.* **23**:100–103 (1983).
13. Kubota, S., and J. T. Yang. Oligopeptides as potential antiaggregation agents for deoxyhemoglobin S. *Proc. Natl. Acad. Sci. USA* **74**:5431–5434 (1977).
14. Gorecki, M., J. R. Voltano, and A. Rich. Peptide inhibitors of sickle hemoglobin aggregation: effect of hydrophobicity. *Biochemistry* **19**:1564–1568 (1980).
15. Franklin, I. M., R. I. Cotter, R. C. Cheetham, J. F. Pardon, A. J. Hale, and E. R. Huehns. A potent new dipeptide inhibitor of cell sickling and haemoglobin S gelation. *Eur. J. Biochem.* **136**:209–214 (1983).
16. Schroeder, W. A., and T. H. J. Huisman. *The Chromatography of Hemoglobin.* Marcel Dekker, New York (1980).
17. Hofrichter, J., P. D. Ross, and W. A. Eaton. Supersaturation in sickle cell hemoglobin solutions. *Proc. Natl. Acad. Sci. USA* **73**:3035–3039 (1976).
18. Cohn, E. J., and J. D. Ferry. Interactions of proteins with ions and dipolar ions, in *Proteins, Amino Acids and Peptides as Ions and Dipolar Ions* (E. J. Cohn and J. T. Edsal, eds.). Reinhold Publishing Corp., New York 586–619 (1943).
19. Schwartz, R. S., K. Kleman, and B. Lubin. Inhibition of sickling in vitro using phenylalanine-loaded liposomes. *Clin. Res.* **31**:323A (1983).
20. Kumpati, J. Inhibition of in vitro sickling by liposome-mediated transport of amino acids into intact human red blood cells. *Biochem. Biophys. Res. Commun.* **105**:482–487 (1982).
21. Gorecki, M., C. T. A. Acquaye, M. Wilchek, J. R. Voltano, and A. Rich. Antisickling activity of amino acid benzyl esters. *Proc. Natl. Acad. Sci. USA* **77**:181–185 (1980).
22. Abraham, D. J., M. F. Perutz, and S. E. V. Phillips. Physiological and x-ray studies of potential antisickling agents. *Proc. Natl. Acad. Sci. USA* **80**:324–328 (1983).
23. Johnson, M., and H. Zeidan. Probable binding region of small hydrophobic molecules on hemoglobin spin label-induced nuclear magnetic relaxation. *Biochim. Biophys. Acta* **744**:193–199 (1983).
24. Russu, I. M., C. Dalvit, A. K.-L. C. Lin, C.-P. Yang, and C. Ho. A proton nuclear magnetic resonance investigation of the antisickling activity of L-amino acids and related compounds. *Biophys. J.* **45**:371a (1984).
25. Sunshine, H. R., J. Hofrichter, W. A. Eaton. Requirements for therapeutic inhibition of sickle haemoglobin gelation. *Nature* **275**:238–240 (1978).
26. Brittenham, G. M., A. N. Schechter, and C. T. Noguchi. Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes. *Blood* **65**:183–189 (1985).

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