either case. However, *his*4-713 is suppressed to a $His^+$ phenotype because of the ability of the *his*4-713 mutant protein to form an active dimer with the small amount of full-length protein produced as a result of suppression, whereas the *his*4-712 mutant protein cannot form such a dimer. The relative positions of *his*4-712 and *his*4-713 within the *his*4 gene support this explanation. The *his*4-713 mutation is only 134 base pairs from the end of the *his*4 gene and can make a protein that is 95 percent the size of wild-type. The *his*4-712 is more than 1000 base pairs from the end of the gene and would make only a small fragment of the *his*4 protein (less than 62 percent the size of the wild-type protein).

Another explanation for the difference in suppressor efficiency of identical four-base codons could be the effect of "context" (*20*). Studies of suppression of *his*D mutations in *S. typhimurium* suggest that the codons in the neighborhood of a suppressible mutation may affect the efficiency of suppression (*21*). A UAG nonsense mutation in *his*D was not suppressed by a known amber tRNA suppressor. A selection for suppression uncovered a strain that contained a single base change in a codon immediately adjacent to the original UAG mutation. The interpretation of these results was that the amber tRNA suppressor could not be accommodated in a side-by-side interaction with the original neighboring tRNA during translation as a result of steric hindrance. More efficient suppression of the UAG mutation occurs in the revertant because the codon adjacent to the UAG has been altered so that it can be read by a tRNA species that does not interfere with the suppressor tRNA. In a similar way the failure of some suppressors of *his*4-713 to suppress *his*4-712 could be related to the codons flanking the *his*4-712 mutation.

A third explanation for the different suppressibility patterns of *his*4-713 and *his*4-712 is that some suppressors of *his*4-713 do not act at the level of suppression of the CCCU codon but at another four-base codon, within a limited distance, which is not present in the *his*4-712 region. For example, a +1 mutation in the *his*D gene of *S. typhimurium* is suppressed at a site downstream from the original mutation by a suppressor of four-base codons (*21*). Although the four-base codon suppressed was not the one affected by the mutation, the suppression restored the reading frame, permitting expression of the gene. Since a nonsense codon (UGA) immediately follows the *his*4-713 mutation, no suppression can occur distal to the mutation as a result of premature termination of translation. However, "indirect" suppression could still occur at a four-base codon proximal to the *his*4-713 frameshift site, which would restore the normal reading frame.

There may be reading of nontriplet codons even in wild-type yeast strains. Frameshift mutations both +1 and −1 in a monotonous run of T's in the *oxi*-1 gene of the mitochondrion have a leaky phenotype (*22*). The leaky phenotypes could result from nontriplet reading by normal tRNA's. Several reports concerning this phenomenon in bacteria suggest that nontriplet codons are read at low levels both in vitro and in vivo (*23–25*). In vitro translation studies indicate that specific wild-type tRNA's are responsible for the level of nontriplet reading (*24*). The translation of nontriplet codons in vivo may be important for the growth of some bacteriophages (*25*). No evidence of a physiological role for nontriplet reading has been reported in eukaryotes.

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## Influence of Calcium Ion on the Binding of Fibrin Amino Terminal Peptides to Fibrinogen

Abstract. *The affinity of the amino terminal tetrapeptide of the β chain of fibrin, Gly-His-Arg-Pro, for fibrinogen dramatically increases in the presence of 2 millimolar calcium ion. In contrast, there is no significant increase in the affinity of peptides beginning with the amino terminal sequence of the fibrin α chain, Gly-Pro-Arg, in the presence of calcium ions, although the number of binding sites increases. In the latter case, the increased number of sites is due to the α chain analogs binding to the site ordinarily occupied by the β chain analogs. These results indicate that structures at the amino terminus of the fibrin β chain play a more important role in fibrin polymerization when calcium ions are present.*

The formation of a fibrin clot involves removal of small polar peptides (fibrinopeptides A and B) from the amino termini of the α and β chains of fibrinogen by thrombin, polymerization of the resultant fibrin monomers to form a noncovalently bound gel, and covalent cross-linking in the presence of activated factor XIII and calcium ion. Peptides beginning with the sequence Gly-Pro-Arg . . . (Gly, glycine; Pro, proline; Arg, arginine), which corresponds to the fibrin α chain amino terminus that is unveiled when thrombin removes fibrinopeptide A, bind to fibrinogen and inhibit the polymerization of fibrin (*1*). The peptide Gly-His-Arg-Pro (His, histidine), the first four amino acids of the fibrin β chain exposed by the release of fibrinopeptide B, binds to separate sites on fibrinogen and does not inhibit polymerization (*2*). Both types of peptide bind to


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Table 1. Effect of calcium on the binding of various peptides to human fibrinogen; $K$ is the association constant and the corresponding standard error determined by least-squares analysis; $N$ is the number of binding sites and the corresponding standard errors determined by least-squares analysis. Differences are significant at $P \leq .05$.

| Peptide | 2 m$M$ $CaCl_2$ present | $CaCl_2$ absent | $P$ |
|---|---|---|---|
| [$^{14}$C]Gly-His-Arg-Pro | | | |
| $K$ | $6.39 \times 10^4 \pm 1.6 \times 10^3$ | $6.84 \times 10^3 \pm 1.31 \times 10^3$ | $< .05$ |
| $N$ | $1.72 \pm 0.09$ | $1.61 \pm 0.18$ | $> .50$ |
| [$^{14}$C]Gly-Pro-Arg-Val | | | |
| $K$ | $1.26 \times 10^4 \pm 9 \times 10^2$ | $9.02 \times 10^3 \pm 1.27 \times 10^3$ | $> .10$ |
| $N$ | $2.78 \pm 0.09$ | $1.71 \pm 0.13$ | $< .01$ |
| [$^{14}$C]Gly-Pro-Arg-Pro | | | |
| $K$ | $4.90 \times 10^4 \pm 4.2 \times 10^3$ | $3.79 \times 10^4 \pm 5.2 \times 10^3$ | $> .1$ |
| $N$ | $3.09 \pm 0.07$ | $2.31 \pm 0.09$ | $< .01$ |
| [$^{14}$C]Gly-Pro-Arg-Pro* | | | |
| $K$ | $7.26 \times 10^4 \pm 1.01 \times 10^4$ | $6.55 \times 10^4 \pm 4.8 \times 10^3$ | $> .5$ |
| $N$ | $1.79 \pm 0.06$ | $1.71 \pm 0.03$ | $> .3$ |

*With a 20-fold excess of nonradioactive Gly-His-Arg-Pro.

fragment D of fibrinogen, the plasmin-derived fragment that approximates the terminal domain. These results are consistent with earlier notions that the fibrin amino terminal regions exposed by fibrinopeptide release may be important binding sites for fibrin polymerization (*3*).

The importance of calcium ions to the structural integrity and function of fibrinogen has become increasingly clear. Calcium ions bind to fibrinogen (*4*), stabilize it against denaturation by heat (*5*) and acid (*6*), and limit the digestion of both fibrinogen and fibrin by proteases such as trypsin (*7*) and plasmin (*8*). Calcium ions accelerate the conversion of fibrinogen into fibrin (*9*) by increasing the rate of fibrin monomer polymerization (*10*). It should also be mentioned that calcium ions are essential for the activity of factor XIII in fibrin cross-linking (*11*).

Accordingly, we undertook a study of the effect of calcium ions on the binding of various synthetic peptides to fibrinogen to see whether any of these interactions were influenced by its presence. Binding affinity and number of sites were determined by equilibrium dialysis, essentially as described (*2*), both in the presence and absence of 2 m$M$ calcium ion.

The binding affinity of the amino terminal tetrapeptide of the fibrin β chain, Gly-His-Arg-Pro, was dramatically strengthened in the presence of 2 m$M$ calcium ion (see Table 1 and Fig. 1A), the association constant increasing from $K = 6.8 \times 10^3 M^{-1}$ to $K = 6.4 \times 10^4 M^{-1}$. The number of binding sites was not changed. In contrast, the peptide Gly-Pro-Arg-Val (Val, valine), the first four amino acids at the amino terminus of the fibrin α chain, showed virtually no increase in affinity when calcium was present although the number of binding sites increased from $N = 1.7$ to $N = 2.8$ (Table 1 and Fig. 1B). Similarly, calcium ions increased the number of apparent fibrinogen binding sites for Gly-Pro-Arg-Pro from $N = 2.3$ to $N = 3.1$ without any significant increase in the association constant (Table 1 and Fig. 1C).

These results indicate that calcium ions were enhancing the affinity of the Gly-His-Arg-Pro binding site. The increase in the number of sites for the α chain analogs, beginning with Gly-Pro-Arg . . . , was due to these peptides fitting into the Gly-His-Arg-Pro binding site as a consequence of the increased affinity of that site, as shown by equilibrium dialysis both in the presence and absence of a 20-fold excess of nonradioactive Gly-His-Arg-Pro with and without 2 m$M$ calcium ion. Thus, excess nonradioactive Gly-His-Arg-Pro virtually eliminates the increase in the number of binding sites for [$^{14}$C]Gly-Pro-Arg-Pro due to calcium ion (Table 1 and Fig. 1D).

We interpret these results as follows. There is a distinct binding site for each of the amino terminal peptides of the fibrin α and β chains in each fragment D portion (that is, the terminal domain) of fibrinogen (*12*). The influence of calcium is predominantly on the Gly-His-Arg-Pro (β chain type) binding site. In the ab-

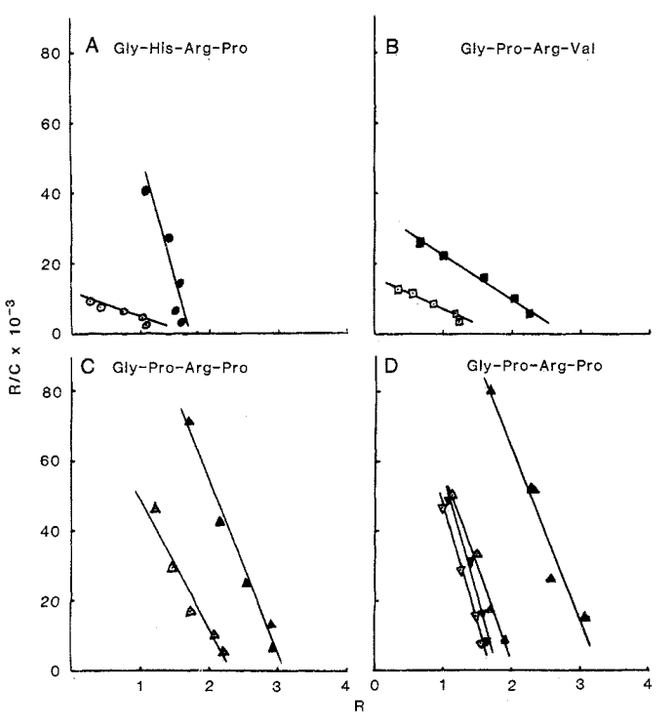


Fig. 1. Effect of calcium on the binding of various radioactive peptides to human fibrinogen. Calcium-free fibrinogen solutions were prepared by dissolving human fibrinogen to a concentration of 13 mg/ml in a solution of 0.3$M$ NaCl, 0.005$M$ imidazole buffer ($p$H 7.2) and 0.005$M$ EGTA. The solutions were dialyzed three times against 0.2$M$ NaCl containing 0.01$M$ imidazole ($p$H 7.2); hirudin (Sigma, grade IV, 1400 unit/mg) was added to a final concentration of 0.05 mg/ml. Portions of the fibrinogen solution (0.8 ml) were then dialyzed for 10 hours against 5 ml of various peptide solutions prepared in 0.2$M$ NaCl, 0.01$M$ imidazole ($p$H 7.2), both in the presence (closed symbols) and absence (open symbols) of 2 m$M$ calcium chloride. Binding of radioactive peptides was determined by liquid scintillation counting of duplicate portions (0.2 ml) from the inside and outside of each bag after 10 hours of equilibration on a mechanical shaker. (A) [$^{14}$C]Gly-His-Arg-Pro binding; (●) 2 m$M$ $CaCl_2$; (○) no $CaCl_2$. (B) [$^{14}$C]Gly-Pro-Arg-Val binding; (■) 2 m$M$ $CaCl_2$; (□) no $CaCl_2$. (C) [$^{14}$C]Gly-Pro-Arg-Pro binding; (▲) 2 m$M$ $CaCL_2$; (△) no $CaCl_2$. (D) [$^{14}$C]Gly-Pro-Arg-Pro binding; (▲) 2 m$M$ $CaCl_2$; no Gly-His-Arg-Pro. (△) No $CaCl_2$, no Gly-His-Arg-Pro; (▼) 2 m$M$ $CaCl_2$, 20-fold excess of nonradioactive Gly-His-Arg-Pro; (△) no $CaCl_2$, 20-fold excess of nonradioactive Gly-His-Arg-Pro. $R$, number of moles of peptide bound per mole of fibrinogen. $C$, concentration of unbound peptide. All studies were done at room temperature (22°C). None of the peptides studied bind detectably to control proteins (*1*, *2*). Accordingly, no corrections for nonspecific binding were required.

sence of calcium, this site is apparently much less accessible. Calcium has no influence on the Gly-Pro-Arg (α chain type) site. The increase in the number of sites for Gly-Pro-Arg-Pro in the presence of calcium is attributable to its binding to the β chain type site. We had demonstrated through competitive binding studies that Gly-Pro-Arg-Pro binds weakly to the Gly-His-Arg-Pro binding site, in addition to its major binding at the two Gly-Pro-Arg type sites (*2*). Indeed, in the absence of calcium, the binding of Gly-Pro-Arg-Pro to the Gly-His-Arg-Pro binding site is so weak that only two Gly-Pro-Arg type binding sites are observed in a Scatchard plot. When calcium ions are present, the affinity of the Gly-His-Arg-Pro binding site for both Gly-His-Arg-Pro and Gly-Pro-Arg type peptides increases. As a consequence, the Scatchard plot extrapolates to more than two Gly-Pro-Arg-Pro binding sites.

The role of fibrinopeptide B release in fibrin polymerization has been the subject of considerable speculation. Because fibrinopeptide A is released by thrombin faster than fibrinopeptide B, it was long ago proposed that removal of fibrinopeptide A is responsible for an end-to-end polymerization producing intermediate polymers, while removal of fibrinopeptide B results in lateral aggregation of these intermediate polymers (*13*). Subsequently it was shown that lamprey fibrinogen could be clotted by bovine thrombin, whch removes only fibrinopeptide B, indicating an important function for fibrinopeptide B release in that species (*14*). Indeed, at low temperatures (< 25°C), even human fibrinogen can be clotted by the selective removal of fibrinopeptide B although clotting was not observed at 37°C unless fibrinopeptide A was also removed. Our finding that calcium increases the affinity of Gly-His-Arg-Pro by almost tenfold indicates that the true physiological circumstances surrounding fibrin formation require the presence of calcium ions. In the past much of the experimentation on the roles of fibrinopeptide release and fibrin formation have been conducted in the absence of calcium ions (*13–15*).

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## Induction of Hemoglobin Accumulation in Human K562 Cells by Hemin Is Reversible

Abstract. *Twenty micromolar hemin causes no change in the rate of division of K562 cells but results in accumulation of 11 to 14 picograms of embryonic and fetal hemoglobins per cell. This effect is reversible, and hemoglobin induction in response to hemin, and loss of hemoglobin upon removal of hemin, can be cyclically repeated. The cells can be indefinitely subcultured in the presence of the inducer. Thus, the control of hemoglobin levels in K562 cells does not depend on irreversible differentiation.*

The mechanisms that control hemoglobin biosynthesis have been investigated in studies of animal models of hemoglobin "switching," erythroid cells in culture, and subcellular systems (*1*). During the last decade the murine Friend erythroleukemia cell line has been studied as a model system that can be induced by various chemicals to undergo erythroid differentiation and to accumulate hemoglobin (*2*). Analyses of cultured human erythroid cells have been restricted by their limited lifetime in vitro and stringent growth requirements. Recently, the K562 human cell line, originally isolated from the pleural effusion of a patient with chronic myelogenous leukemia (*3*) has been investigated as a potential human analog of the Friend cell. K562 cells can be induced by 50 to 100 μ*M* hemin to form embryonic and fetal hemoglobins (*4–6*). We have examined the growth characteristics and accumulation of hemoglobin in K562 cells grown with low concentrations of hemin. We report here that human K562 cells can be caused to accumulate human embryonic and fetal hemoglobins without irreversible erythroid differentiation or commitment.

The K562 cells were grown in suspension in RPMI 1640 medium containing 10

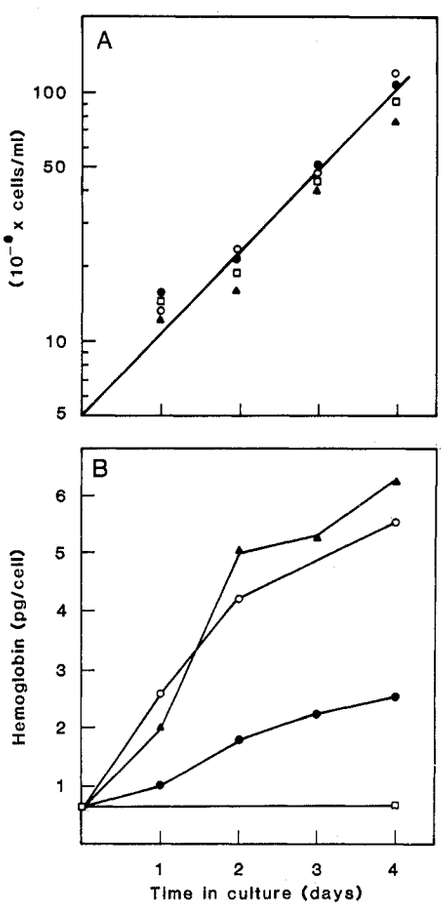


Fig. 1. (A) Cell counts from K562 cultures initiated at $5 \times 10^4$ cells per milliliter and treated with 10 μ*M* (●), 20 μ*M* (○), or 30 μ*M* (▲) hemin, or with no added hemin (□). A stock solution of 600 μ*M* hemin was prepared by adding 0.3 ml of 1*N* NaOH to 19.56 mg of hemin, and then adding 0.3 ml of 0.5*M* tris base, 2.5 ml of cell growth medium, and 0.35 ml of 1*N* HCl. The solution was sterilized by filtration. (B) Accumulation of hemoglobin in K562 cells induced with 10 μ*M* (●), 20 μ*M* (○), or 30 μ*M* (▲) hemin, or with no added hemin (□). Hemoglobin was measured spectrophotometrically in cell lysates as described (*7*).


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