AN ARCHETYPE CORRELATION BETWEEN BACTERIAL RUBREDOXIN AND BOTH BACTERIAL AND PLANT FERREDOXINS

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The non-heme iron protein rubredoxin, isolated originally from Clostridium pasteurianum, replaces ferredoxin in certain enzymatic reactions (Lovenberg et al, 1965). Another rubredoxin, obtained from Micrococcus aerogenes, was used in a study of the inorganic binding sites (Bachmeyer et al, 1967). The structure of this second compound consists of a linear chain of fifty-three amino acids (Bachmeyer et al, 1968a, 1968c). A third rubredoxin, derived from Peptostreptococcus elsdenii, possesses a similar array of fifty-two residues (Bachmeyer et al, 1968b). A preliminary investigation now leads to the conclusion that the rubredoxins may exhibit an internal duplication, and are possibly related to the plant ferredoxins.

The sequences of various ferredoxins isolated from different sources--bacteria [Clostridium pasteurianum (Tanaka et al, 1964, 1966; Benson et al, 1968), Clostridium butyricum (Benson et al, 1966), Micrococcus aerogenes (Tsunoda et al, 1967)]; plants [spinach (Matsubara et al, 1968b), alfalfa (Keresztes-Nagy et al, 1966, 1968)]; green alga [Scenedesmus (Sugeno, 1968)]--were established in parallel work. Comparisons between the ferredoxins are very useful from the viewpoint of functional (Malkin et al, 1967; Matsubara, 1968a) and evolutionary relationships (Eck et

al, 1966, Jukes, 1966). A prototype of twenty-nine residues, by undergoing lengthening by recombination, would afford the known bacterial ferredoxins (Matsubara et al, 1969b). Algal, bacterial, and plant ferredoxins have similar sequences, which is suggestive of a common archetype origin. Chromatium ferredoxin, derived from a photosynthetic anaerobic bacteria, appears intermediate between the nonphotosynthetic bacteria and green plants (Sasaki et al, 1967).

In view of the biological interchange between the bacterial ferredoxins and rubredoxins, an "active site" or a residue homology might be expected between these proteins. At present, one is limited to M. aerogenes, since sequence work is unavailable for other ferredoxin-rubredoxin pairs. With this consideration in mind, a tentative arrangement involving these two compounds is shown in Fig. 1. For ferredoxin, the numbering scheme, and the gaps, with minor exceptions, follow earlier examples (Matsubara et al, 1967). An interesting pattern of par-

FERREDOXIN

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1                   5                  10                  15                  20                  25
Ala Tyr Val Ile Asn — Asp Ser Cys Ile Ala Cys Gly Ala Cys Lys Pro Glu Cys Pro Val Asn — Ile Gln Gln Gly — Ser
Ile Tyr Ala Ile Asp Ala Asp Ser Cys Ile Asp Cys Gly Ser Cys Ala Ser Val Cys Pro Val Gly Ala Pro Asn Pro Glu Asp —
27                  32                  37                  42                  47                  52

Base
Differences
2  0  1  0  1  —  0  0  0  0  1  0  0  1  0  2  1  1  0  0  0  2  —  2  2  1  1  —  —

Minimum base differences per codon:  0.72
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RUBREDOXIN

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                1                   5                  10                  15                  20                  25
—  —  —  Met Gln Lys Phe Glu Cys Thr Leu Cys Gly Tyr Ile Tyr Asp Pro Ala Leu Val Gly Pro Asp Thr Pro Asp Gln Asp Gly Ala Phe
Glu Asp Val Ser Glu Asn Trp Val Cys Pro Leu Cys Gly Ala Gly Lys Glu Asp Phe Glu Val Tyr Glu Asp — — — — — — — —
30                  35                  40                  45                  50

Base
Differences
—  —  —  2  1  1  2  1  0  1  0  0  0  2  2  2  1  2  2  2  0  2  2  0  —  —  —  —  —  —  —  —

Minimum base differences per codon:  1.19
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Figure 1. The amino acid sequences of both Micrococcus aerogenes ferredoxin and rubredoxin aligned for maximum internal and external correspondence. Identical positional residues are underscored within each peptide; residues common to both peptides are enclosed by boxes.

tial correspondence emerges by allowing the initial cysteine in the rubredoxin to match the equivalent residue in the ferredoxin. Unfortunately, the evidence appears marginal for internal repetition in rubredoxin, since the MBDC value is high (1.19), thus a direct relationship is not evident for the two bacterial proteins. These points merit reevaluation when additional sequence data is in hand for other ferredoxin-rubredoxin sets.

Attention has been called to similarities in certain regions of spinach and *Clostridium butyricum* ferredoxin, whose compatibility was strengthened by the employment of deletions. A generalized presentation is given in Fig. 2. Thirteen residues in *M. aerogenes* ferredoxin are identical with similar ones in spinach ferredoxin. An alternative and larger scheme for the ferredoxins begins at the amino terminus and involves a doubling mechanism (Matsubara et al, 1969).

SPINACH FERREDOXIN (31-89) AND M. AEROGENES FERREDOXIN (1-54)

	31				35					40					45					50					55					60		
	Glu	Gly	Ile	Asp	Leu	Pro	Tyr	Ser	Cys	Arg	Ala	Gly	Ser	Cys	Ser	Ser	Cys	Ala	Gly	Lys	Leu	Lys	Thr	Glv	Ser	Leu	Asn	Gln	Asp	Asp	Gln	Ser
	Ala	Tyr	Val	—	Ile	Asn	Asp	Ser	Cys	Ile	Ala	—	—	Cys	Gly	Ala	Cys	Lys	Pro	Glu	Cys	—	Pro	Val	Asn	Ile	Gln	Gln	Gly	Ser	Ile	Tyr
	1					5					10							15						20					25			
Base Differences	1	2	1	—	1	2	1	0	0	1	0	—	—	0	1	1	0	2	2	1	2	—	1	1	1	1	2	0	1	2	2	1

	63					68					73					78						83					88	
	Phe	Leu	Asp	Asp	Asp	Gln	Ile	Asp	Glu	Gly	Trp	Val	Leu	Thr	Cys	Ala	Ala	—	Tyr	Pro	Val	Ser	Asp	Val	Thr	Ile	Glu	Thr
	Ala	Ile	Asp	Ala	Asp	Ser	Cys	Ile	Asp	—	—	Cys	Gly	Ser	Cys	Ala	Ser	Val	Cys	Pro	Val	Gly	Ala	Pro	Asn	Pro	Glu	Asp
		30					35							40					45					50				
Base Differences	2	1	0	1	0	2	2	2	1	—	—	2	2	1	0	0	1	—	1	0	0	1	1	2	1	2	0	2

Minimum base differences per codon: 1.08

Figure 2. Similarities between spinach ferredoxin and *Micrococcus aerogenes* ferredoxin. Identical positional residues are underscored within each peptide.

In Fig. 3, spinach and *Scenedesmus* ferredoxins are compared with *M. aerogenes* rubredoxin. Most significantly, the

SPINACH FERREDOXIN (13-36) AND M. AEROGENES RUBREDOXIN (1-23)

				15				20				25				30				35				
Asn	Val	Glu	Phe	Gln	Cys	Pro	Asp	Asp	Val	Tyr	Ile	Leu	Asp	Ala	Ala	Glu	Glu	Glu	Gly	Ile	Asp	Leu	Pro	
Met	Gln	Lys	Phe	Glu	Cys	Thr	Leu	Cys	Gly	Tyr	Ile	Tyr	Asp	Pro	Ala	Leu	Val	—	Gly	Pro	Asp	Thr	Pro	
1				5					10					15						20				
Base Differences																								
2	2	1	0	1	0	1	2	2	1	0	0	2	0	1	0	2	1	—	0	2	0	2	0	

Minimum base differences per codon: 0.96

SCENEDESMUS FERREDOXIN (13-36) AND M. AEROGENES RUBREDOXIN (1-23)

				15				20				25				30				35				
Asn	Gln	Thr	Ile	Glu	Cys	Pro	Asp	Asp	Thr	Tyr	Ile	Leu	Asp	Ala	Ala	Glu	Glu	Ala	Gly	Leu	Asp	Leu	Pro	
Met	Gln	Lys	Phe	Glu	Cys	Thr	Leu	Cys	Gly	Tyr	Ile	Tyr	Asp	Pro	Ala	Leu	Val	—	Gly	Pro	Asp	Thr	Pro	
1				5					10					15						20				
Base Differences																								
2	0	1	1	0	0	1	2	2	2	0	0	2	0	1	0	2	1	—	0	1	0	2	0	

Minimum base differences per codon: 0.87

Figure 3. Similarities between spinach and Scenedesmus ferredoxins with Micrococcus aerogenes rubredoxin. Identical positional residues are underscored within each peptide.

correlation commences in the initial portions of the plant and algal ferredoxins. The MBDC values (0.96 and 0.87) are slightly lower than related numbers derived for selected regions of spinach ferredoxin vs. M. aerogenes ferredoxin. The fit suggests rubredoxin is an important relic, which is definitely associated with the plant ferredoxins. Rubredoxin will no doubt serve as a guide to future phylogenetic work in this area, for it appears to bridge both the bacterial and plant ferredoxins.

Finally, two additional observations need mentioning: first, the residue reciprocity between the algal, bacterial, and plant ferredoxins with rubredoxin does not support one suggestion as to their genetic evolution (Eck et al, 1966); and, second, the division of the ferredoxins into three groups--green plants and algae, photosynthetic bacteria, and nonphotosynthetic bacteria (Sasaki et al, 1967)--must be revised to include the rubredoxins.

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