Structural Basis for the Species Selectivity of a Fibrin-Specific Monoclonal Antibody†

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ABSTRACT: The structural determinant underlying the species specificity of a monoclonal anti-fibrin antibody (59D8) is the leucyl residue at position 5 in β-chains of human fibrin. Anti-fibrin antibody 59D8 which had been elicited by immunization with human β(1–7) peptide, Gly-His-Arg-Pro-Leu-Asp-Lys, binds to human and canine fibrins but not to bovine, ovine, or porcine fibrins. A comparison of the available amino acid sequence data suggested that the ability of anti-fibrin antibody 59D8 to discriminate among various fibrin β-chains might be due to the amino acid at position 5. This was confirmed by competitive inhibition studies using synthetic fibrin-like peptides and determination of the amino acid sequences of the N-termini of ovine and porcine fibrin β-chains. Edman degradation employing *o*-phthalaldehyde blocking permitted use of fibrin monomer rather than its separated constituent polypeptide chains. The same sequencing strategy was used to obtain partial sequence data for the α-chains of bovine, ovine, and porcine fibrin.

Fibrin-specific antibodies have been sought as potentially useful clinical agents to detect thrombi in vivo and to evaluate various thrombolytic states. Anti-fibrin antibodies have been studied as agents for the detection of solid tumors by localization of a fibrin capsule formed by the host in response to the growing tumor (Day et al., 1958; Bale et al., 1960, 1980). The major problem in these attempts has been cross-reactivity of anti-fibrin antibodies with fibrinogen which is present at a level of 3–4 mg/mL in plasma. With a single exception (Bosnjakovic et al., 1977), past efforts to produce fibrin-specific antibodies have been hindered by the immunological similarity between fibrin and its soluble precursor, fibrinogen.

Fibrinogen is the least soluble among the plasma proteins [reviewed by Doolittle (1973) and Mosesson & Doolittle (1983)]. With a molecular weight of 340 000, it possesses a 2-fold symmetry which arises from three pairs of nonidentical polypeptide chains designated Aα, Bβ, and γ. At the site of thrombosis, the coagulation cascade is activated to generate thrombin, which enzymatically cleaves two pairs of polar peptides from fibrinogen, fibrinopeptides A and B, to yield fibrin monomers. These monomers, being much less soluble, spontaneously polymerize into a gel network. Almost simultaneously, the fibrin clot is stabilized by factor XIIIa which introduces interchain N^{ϵ}-(γ-glutamyl)lysyl bonds.

Characteristically, anti-fibrin sera when depleted of anti-fibrinogen reactivity fail to retain anti-fibrin immunoreactivity. Rather than using fibrin itself as antigen, Cierniewski et al. (1982) isolated a peptide containing the γ-chain cross-link site to produce rabbit antisera which bound selectively to D dimer, a fibrin degradation product, but not fibrinogen. By using a different immunochemical strategy, we obtained polyclonal antibodies which bound to fibrin even in the presence of plasma concentrations of fibrinogen (Pacella et al., 1983). These antibodies were elicited by immunization with synthetic decapeptides which corresponded to the N-termini of fibrin α- and β-chains which are exposed following thrombin cleavage.

Monoclonal anti-fibrin antibodies which bind to human fibrin but not to fibrinogen have been recently prepared and characterized. Using synthetic peptide antigens, we (Matsueda et al., 1983; Hui et al., 1983) obtained fibrin-specific monoclonal antibodies 59D8, 64C5, and 55D10, which recognize the N-terminus of the fibrin β-chain. Analogous antibodies have been described by Scheefers-Borchel et al. (1984), who used a synthetic hexapeptide from the N-terminus of fibrin α-chains. By using fragments of fibrin as antigens, Kudryk et al. (1984) described a monoclonal antibody which binds to the N-terminus of fibrin β-chains. Elms et al. (1983) produced a monoclonal antibody which binds specifically to cross-linked fibrin.

To permit selection of a suitable in vivo model for thrombus detection and the consequent evaluation of our anti-fibrin monoclonal antibodies, we examined the species specificity of anti-fibrin antibody 59D8 and propose an explanation for this selectivity.

Materials and Methods

Preparation of Fibrin Monomer. The procedure of Brossted et al. (1977) was used with modifications as described below. Canine, bovine, ovine, and porcine fibrinogens were obtained from Sigma Chemical Co. (lots 71F9345, 12F9315, 70F9320, and 71F9360, respectively). Each fibrinogen (160 mg of protein) was dissolved in 5 mL of water which contained 4600 kallikrein inhibition units (KIU) of aprotinin and 60 mg of iodoacetamide, centrifuged to remove insoluble materials, and then mixed with 5.0 mL of 0.05 M ethylenediaminetetraacetic acid (EDTA), pH 7.0, which contained 10 NIH units of human thrombin (Sigma, lot 73F9459). Within minutes at 37 °C, clots formed and were retrieved with wooden sticks. The clots were washed in saline, minced, and dissolved in 1 M NaBr/0.05 M NaOAc, pH 5.5. The concentration of each fibrin monomer solution was determined by the absorbance at 280 nm assuming $E^{0.1\%} = 1.5$.

Solid-Phase Peptide Synthesis. β(1–8) amide and two position 5 analogues were prepared as fibrin-like inhibitors of antibody 59D8. Starting with *p*-methylbenzhydrylamine (MBHA) resin (Matsueda & Stewart, 1981), each *tert*-butyloxycarbonyl-amino acid (Boc-amino acid) was coupled

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sequentially by using a Beckman 990C synthesizer to give Gly-His(Tos)-Arg(Tos)-Pro-Xxx-Asp(Bzl)-Lys(ClZ)-Lys-(ClZ)-NH-MBHA resin where Xxx was Leu, Ala, or Tyr-(BrZ) in each of three separate syntheses, Tos is tosyl, Bzl is benzyl, and Z is benzyloxycarbonyl. Following anhydrous hydrogen fluoride treatment (Stewart & Young, 1984), the peptides were gel filtered on Bio-Gel P-2 columns in 1 M HOAc and purified by Whatman DE-52 ion-exchange chromatography using a linear gradient of NH_4OAc, pH 5.5, from 0.01 to 0.25 M. Fractions containing peptides judged pure by thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) were lyophilized, dissolved in water, and then analyzed following HCl hydrolysis. Thus, the concentration of each peptide and its amino acid composition were determined simultaneously. The compositions were as follows: Leu^5-β(1–8) amide gave Asp 0.99, Pro 1.14, Gly 0.95, Leu 0.96, His 0.96, Lys 1.94, and Arg 1.04; Ala^5-β(1–8) amide gave Asp 1.06, Pro 1.09, Gly 0.93, Ala 0.96, His 0.97, Lys 2.00, and Arg 0.97; Tyr^5-β(1–8) amide gave Asp 1.00, Pro 0.91, Gly 0.92, Tyr 0.88, His 0.97, Lys 2.00, and Arg 1.03.

Cross-Reactivity of Antibody 59D8 with Fibrin Monomer of Different Species. A solid-phase radioimmunometric assay was used to assess the cross-reactivity of monoclonal antibody 59D8. This anti-fibrin antibody was elicited by immunization with a synthetic peptide which corresponded to the first seven amino acids from the amino terminus of the β-chain in human fibrin (Hui et al., 1983). Culture supernatants containing hybridoma protein 59D8 were titered to 50% of maximum binding. As antigen, aliquots of serially diluted (1:4) fibrin monomer dissolved in 1 M NaBr buffered with 0.05 M Na-OAc, pH 5.5, were placed in wells of poly(vinyl chloride) microtiter plates and incubated for 3 h at 25 °C. As homologous proteins were dissolved in a buffer of identical composition, we assumed that the quantity of fibrin adsorbed was proportional to protein concentrations. Each well was then treated for 30 min with 25% γ-globulin-free horse serum to minimize nonspecific binding. After wells were washed, aliquots of titered 59D8 culture supernatants were then applied to all wells, incubated for 1 h, and then washed thoroughly before addition of ^{125}I-labeled goat anti-mouse second antibody (50000 cpm/well). After exhaustive washing to remove excess radiolabeled second antibody, specifically bound antibody was quantified by scintillation counting (Micromedics Model 4-600).

Specificity of Antibody 59D8 toward Synthetic Fibrin-like Peptides. A solid-phase radioimmunometric assay was used to compare the inhibitory potency of three fibrin-like peptides. As antigen, 25-μL aliquots of 0.05 mg/mL human fibrin monomer dissolved in 1 M NaBr/0.05 M NaOAc, pH 5.5, were placed into individual wells of a microtiter plate and incubated for 18 h at 4 °C. After the antigen-coated wells were treated with 25% horse serum as described above, mixtures of antibody 59D8 and inhibitor peptides were placed in the wells. These solutions were prepared 30 min in advance by mixing equal volumes of serially diluted (1:4) inhibitor peptide in 0.01 M sodium phosphate, pH 7.4, and 0.15 M NaCl containing 0.02% sodium azide (PBSA) and antibody 59D8. The concentration of antibody was chosen to give half-maximal binding in the absence of any inhibitor. After 60 min, the antibody/inhibitor solutions were discarded. The microtiter plates were washed 6 times to remove excess antibody. Specifically bound 59D8 was quantified as described above with radiolabeled second antibody.

Determination of Amino Acid Sequences. Automated Edman degradation was performed on a Beckman 890C sequencer equipped with a cold trap and a Sequemat SC150 sequential controller (Sequemat, Inc., Watertown, MA). A 0.1 M Quadrol single-cleavage program was used (Brauer et al., 1975). At cycles where proline was expected to be amino terminal, *o*-phthalaldehyde was added to the cup in butyl chloride as described in detail elsewhere (Brauer et al., 1984), in order to truncate polypeptides with exposed primary amino groups. This method thus permits the elimination of unwanted sequences in peptide mixtures without the necessity of purification of the target proline-containing peptide. Following *o*-phthalaldehyde treatment, at cycles amino terminal in proline, two successive acid cleavages were required, as cleavage of (phenylthiocarbamyl)prolyl–X bonds is slow (Brandt et al., 1976).

Automated conversion of 2-anilino-5-thiazolinone amino acids to the phenylthiohydantoin (Pth) derivatives with methanol/HCl was performed as described (Margolies et al., 1982). Pth-amino acids were identified and quantified by HPLC on cyanopropyl columns (IBM) using a phosphate buffer system based on the method reported by Hawke et al. (1982).

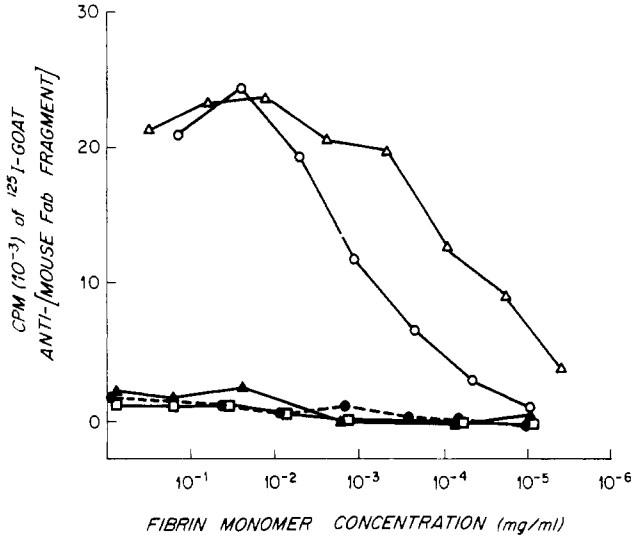

FIGURE 1: Species specificity of monoclonal anti-fibrin antibody 59D8. The direct binding of 59D8 was tested on fibrin monomer obtained from human (open triangles), dog (open circles), cow (closed triangles), pig (closed circles), and sheep (open boxes). Each fibrin monomer dissolved in 1 M NaBr/0.05 M NaOAc, Ph 5.3, was serially diluted in the same buffer and adsorbed to wells of plastic microtiter plates for 3 h. The extent of adsorption was assumed to be proportional to fibrin concentrations which have been plotted on the abscissa. After blocking with 10% γ-globulin-free horse serum containing aprotinin (20 KIU/mL), heparin (20 units/mL), phenylmethanesulfonyl fluoride (3 mM), and NaN_3 (0.02%), monoclonal anti-fibrin antibody 59D8 (10 μg/mL) was placed in each well. After six washes, specifically bound antibody was detected by addition of 50000 cpm of ^{125}I-labeled goat anti-mouse Fab fragment.

RESULTS

Immunospecificity of Monoclonal Anti-fibrin Antibody 59D8. Antibody 59D8 binds effectively to human and dog fibrin monomers, but not significantly to cow, pig, and sheep fibrin monomers as shown in Figure 1.

Edman Degradation Using o-Phthalaldehyde Blockade. To correlate immunoreactivity with structure, N-terminal sequence analysis of fibrin β-chains was performed. As human fibrin β-chain is known to contain proline at position 4 (Iwanaga et al., 1967), Edman degradation using *o*-phthalaldehyde treatment just prior to cycle 4 was used to selectively sequence the β-chain from unfractionated fibrin monomer of each of five different species (Table I). A mixture of three

Table I: Comparison of β-Chain Sequences following Edman Degradation of Fibrin Monomers[a] with o-Phthalaldehyde Treatment[b]

	Pth-amino acids identified (nmol)[c] for Edman degradation cycle									
	1	2	3	4	5	6	7	8	9	10
human[d]	Tyr Gly	Val His Pro	Ala Arg	Pro	Leu	Asp	Lys	Lys	Arg	
canine[e]	Tyr Gly	Thr His Pro	Ala Arg	Pro	Leu	Asp	Lys	Lys	Arg	Glu
bovine[f]	Tyr (8.5) Gly (16.9)	Val (8.5) His (3.0) Pro (5.8)	Ala (8.4) Arg (8.4)	Pro (6.9)	Tyr (7.8)	Asp (2.9)	Lys (10.0)	Lys (10.5)	Lys (10.5)	Glu (4.9)
porcine	Tyr (13.2) Gly (13.0)	Val (12.4) His (9) Pro (9.2)	Ala (11.4) Arg (10.9)	Pro (13)	Tyr (10.8)	Asp (5.6)	Lys (10.4)	Arg (9.45)	Arg (9.5)	Glu
ovine	Tyr (4.7) Gly (8.1)	Val (5.1) His (1.3) Pro (1.4)	Ala (3.0) Arg (2.4)	Pro (1.7)	Tyr (2.6)	Asp (1.9)	Lys (2.6)	Arg (1.2)	Arg (1.5)	Glu (2.1)

[a] Samples of fibrin monomer were obtained by treating fibrinogen from each species with bovine thrombin (5 NIH units/mL) in the presence of 10 mM EDTA. [b] After cycle 3, the sequencer was programmed to treat the fibrin monomer sample with o-phthalaldehyde before continuing with Edman degradation. [c] Following automated conversion, Pth-amino acids were identified by HPLC. [d] Confirms Iwanaga et al. (1967). [e] Confirms Birken et al. (1975). [f] Confirms Cottrell and Doolittle (1976).

Table II: Comparison of α-Chain Sequences after Edman Degradation of Fibrin Monomers[a] with o-Phthalaldehyde Treatment[b]

	Pth-amino acids identified (nmol)[c] for Edman degradation cycle									
	1	2	3	4	5	6	7	8	9	10
human[d]	Tyr Gly	Pro	Arg	Val	Val	Glu	Arg	His	Gln	Ser
canine	Tyr Gly	Pro	Arg	Ile	Val	Glu	Arg	Gln[e]	Gln	Ser
bovine[f]	Tyr (7.7) Gly (8.4)	Pro (7.2)	Arg (5.9)	Leu (7.8)	Val (7.6)	Glu (4.7)	Arg (3.4)	Gln (3.2)	Gln (3.9)	Ser (1.6)
porcine	Tyr (10.6) Gly (11)	Pro (11)	Arg (9.4)	Leu (8.2)	Thr (4.4)	Glu (8.8)	Arg (5.6)	His (4.0)	Gln (3.5)	Ser
ovine	Tyr (6.2) Gly (10.6)	Pro (7.0)	Arg (5.2)	Leu (7.5)	Val (6.2)	Glu (4.8)	Lys (5.9)	Gln (2.4)	Gln (3.1)	Ser (0.9)

[a] Samples of fibrin monomer were obtained by treating fibrinogen from each species with bovine thrombin (5 NIH units/mL) in the presence of 10 mM EDTA. [b] After cycle 1, the sequencer was programmed to treat the fibrin monomer sample with o-phthalaldehyde before continuing with Edman degradation. [c] Following automated conversion, Pth-amino acids were identified by HPLC. [d] Sequence of β-chain confirms report by Iwanaga et al. (1967). [e] Confirms Birken et al. (1975), establishing glutamine at position 8. [f] Confirms Cottrell and Doolittle (1976), establishing positions 8, 9, and 10.

amino acids was found at each cycle prior to o-phthalaldehyde blockade; the yields of Pth-glycine at cycle 1 being greater than expected for a single chain were consistent with the expected sum for amino-terminal glycine occurring in both α- and β-chains. In cycles following o-phthalaldehyde blocking, a single unique Pth-amino acid was found at each step corresponding to the sequence of the β-chain. The data obtained for human and canine fibrin are in agreement with reports by Iwanaga et al. (1967) and Birken et al. (1975), respectively.

The o-phthalaldehyde sequencing strategy was also used to obtain a partial sequence for the α-chains of each fibrin monomer preparation. This was done by treating the fibrin monomer sample with o-phthalaldehyde after cycle 1 rather than cycle 3. These data which have not been previously reported for ovine, bovine, and porcine fibrin are presented in Table II.

Binding of Anti-fibrin Antibody 59D8 to Model Fibrin-like Peptides. To directly assess the contribution of position 5 in the β-chain of fibrin, three fibrin-like peptides were synthesized and used to inhibit the binding of antibody 59D8 to fibrin. To emphasize the similarity between the model peptides and fibrin β-chains, an additional amino acid, Lys, was added and its carboxy group modified as an α-carboxamide to abrogate ionization. As shown in Figure 2, the Leu[5] homologue, β(1–8)

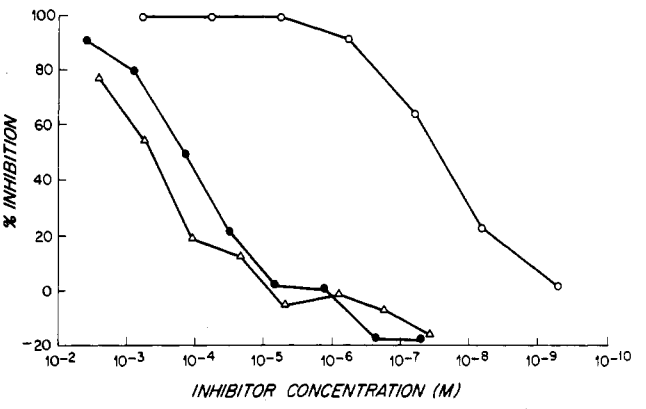

FIGURE 2: Inhibition of anti-fibrin antibody 59D8 binding by synthetic fibrin-like peptides: β(1–8) amide homologue to human β-fibrin (open circles); Tyr[5]-β(1–8) amide homologue to bovine, ovine, and porcine fibrin β-chains (closed circles); and Ala[5]-β(1–8) amide analogue inhibitor peptide (open triangles). As antigen, human fibrin monomer (25 μL, 0.05 mg/mL) was adsorbed to individual wells of plastic microtiter plates. After treatment with 10% γ-globulin-free horse serum, a mixture of anti-fibrin antibody 59D8 and inhibitor peptide was placed into each well. After wells were washed to remove excess anti-fibrin antibody, the specifically bound murine monoclonal antibody was quantified by addition of 50 000 cpm of ^{125}I-labeled goat anti-mouse Fab fragment.

amide, was a potent inhibitor of 59D8 binding to fibrin, as anticipated. By comparison, the Tyr^5 analogue which was used to model the ungulate fibrin β-chains was an ineffective inhibitor, providing only 0.02% relative inhibition. The Ala^5 analogue was also a weak inhibitor (Figure 2).

Discussion

The first seven amino acids of the β-chain in human fibrin, when used as peptide antigen, yielded the hybridoma antibody 59D8. This was done by immunizing a mouse with β(1–7)-Cys-*S*(maleimidobenzoyl)hemocyanin and selecting those monoclonal antibodies which bind to human fibrin even in the presence of a plasma concentration of human fibrinogen (4 mg/mL) but not in the presence of the peptide antigen (Matsueda et al., 1983; Hui et al., 1983).

Since the N-terminal seven amino acids of human (Iwanaga et al., 1976) and canine (Cottrell & Doolittle, 1976) fibrin β-chain are identical, antibody 59D8 was expected to cross-react effectively with dog fibrin monomer. The direct binding assay shown in Figure 1 confirmed this immuno-cross-reactivity and revealed in addition that none of the ungulate fibrins from cow, pig, or sheep were recognized by antibody 59D8.

The available sequence data indicated that of the seven N-terminal positions two might account for the failure of anti-fibrin 59D8 to bind to bovine fibrin monomer. Cottrell and Doolittle (1976) found Tyr-Asx in positions 5 and 6 of bovine fibrin whereas in the human β-chain these two amino acids were Leu-Asp (Iwanaga et al., 1967). To confirm the differences and address the Asp/Asn ambiguity, the *o*-phthalaldehyde (OPA) blocking strategy (Brauer et al., 1984) was used. An unequivocal amino acid sequence can be deduced by Edman degradaton if a peptide mixture is treated with OPA at cycles where proline is N-terminal. Accordingly, a sample of bovine fibrin monomer was sequenced directly as a mixture of α-, β-, and γ-chains for 3 cycles and then treated with OPA to truncate both the α- and γ-chains. When the Edman degradation was resumed at cycle 4, only a single sequence starting with Pth-Pro was detected as indicated in Table I. These data confirmed the Leu/Tyr sequence variation at position 5 and revealed that Pth-Asp rather than Pth-Asn was found at position 6 of the bovine β-chain. These results eliminated the sequence ambiguity, leaving a singular difference at position 5, Tyr (bovine) vs. Leu (human), to explain the nonreactivity of antibody 59D8 toward bovine fibrin monomer.

By using the same sequencing strategy, partial sequences corresponding to the β-chains for human, canine, porcine, and ovine fibrin monomers were obtained (Table I). Taken together, these data suggest that position 5 alone underlies the species selectivity of antibody 59D8 if one assumes that by analogy Gly-His-Arg was the sequence of the first three positions in the β-chains of ovine and porcine fibrins.

To assess the contribution of the sequence variation at position 5 independently, fibrin-like peptides were synthesized as model inhibitors for use in competitive radioimmunometric assays. As shown in Figure 2, Leu^5-β(1–8) amide which corresponded to human β-chain was a potent inhibitor, as expected, providing half-maximal inhibition at 3×10^{-8} M. By contrast, both position 5 analogues containing either Tyr^5 or Ala^5 were ineffective as inhibitors with only 0.02% relative potency. These data established that the amino acid residue in position 5, even out of the context of the entire fibrin molecule, was an important determinant for immunorecognition by antibody 59D8. Furthermore, since the Tyr^5-β(1–8) amide was designed to mimic the N-terminus of ungulate fibrin β-chains, these data confirmed that recognition of Leu^5 was responsible for the species selectivity of antibody 59D8.

Conservative amino acid substitutions in the fibrin β-chains at positions 8 and 9 were also found (Table I), but since these amino acids were not present in the original antigen used for immunization, we assume these differences do not contribute to the species specificity of antibody 59D8.

Partial sequence data for fibrin α-chains are presented in Table II. These data were generated by using the OPA blocking strategy after cycle 1 of each Edman degradation run. It is evident that the amino acid substitutions are conservative at positions 4, 5, and 7 of the α-chains with the exception of position 8 at which either Gln or His was detected. The ungulate α-chains did not share common substitutions except at position 4 where only Leu was found. The data in Table II support the assignment of Gly-His-Arg to β-chains from the mixture sequence shown in Table I, in that these amino acids are not excluded after subtraction of Pro-Arg at positions 2 and 3 of α-chains in Table II (Pth-Arg was recovered at cycle 3 in a yield consistent with two Arg residues). In addition, these data will be particularly useful to correlate the various α-chain sequences with the immunoreactivities of new anti-α-chain monoclonal antibodies which have been elicited by immunization with human α(1–10) fibrin-like peptide (G. R. Matsueda, unpublished results) or the monoclonal antibodies reported by Scheefers-Borchel et al. (1984).

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Isolation and Identification of the Principal Siderophore of the Dermatophyte *Microsporum gypseum*

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ABSTRACT: The dermatophyte *Microsporum gypseum* has been shown to produce two siderophores under conditions of low-iron stress. These compounds have been separated as Fe(III) complexes on silica gel, and the principal siderophore has been identified as ferricrocin using the methods of amino acid analysis, comparative thin-layer chromatography, partial sequencing by gas chromatography–mass spectrometry, ultraviolet spectroscopy, and proton nuclear magnetic resonance spectroscopy of the Al(III) complex.

Since the pioneering discovery by Neilands (1952) of ferrichrome in cultures of the smut fungus *Ustilago sphaerogena*, the chemistry of iron transport by fungi and bacteria has become an important and productive area of research (Neilands, 1973; Emory, 1974; Raymond & Carrano, 1979). Iron is an essential element for all forms of life, and to compensate for the extreme insolubility of Fe(III), the dominant oxidation state of that element in aerobic systems, microorganisms have evolved the capability of producing potent chelating agents, generally called siderophores, to transport iron into the cell where it is released for utilization in various life-essential processes. In fungi, various secondary hydroxamate peptides have been found most commonly as the iron-chelating ligands, while in bacteria, catecholates have proven to be important (Neilands, 1972). The ferrichrome class of iron-transporting compounds, frequently utilized by fungi, are cyclic hexapeptides consisting of three N^{δ}-acyl-N^{δ}-hydroxyl-L-ornithines linked in sequence, with the remaining three amino acids being various sequences of serine, glycine, and, occasionally, alanine.

The availability of iron is a critical factor in determining the pathogenicity of microorganisms invading living hosts, and successful microorganisms must produce iron-transport molecules which can favorably compete with iron chelators present in the host. The ability of mammalian serum to withhold iron from microorganisms is related to the binding of iron by proteins such as transferrin or lactoferrin. Upon invasion, the host response is a reduction of iron concentration in the blood plasma (Cortell & Conrad, 1967; Pekarak et al., 1972).

As early as 1923, it was recognized that serum has an inhibitory effect on dermatophytes (Jessner & Hoffman, 1923). In 1975, King et al. characterized the serum inhibition factor for dermatophyte growth as nondialyzable, fungistatic, and heat-stable at 56 °C for 4 h, and identified the factor as transferrin. They also demonstrated that addition of iron to serum lowered the inhibitory capacity of the serum and that the addition of other metals such as zinc, magnesium, manganese, or copper did not produce the same effect. The same group also showed that no differences existed between the inhibitory activity of serum from normal patients and that from patients with dermatophytosis (Carlisle et al., 1974). Recently, Kerbs et al (1979) demonstrated that deferoxamine methanesulfonate, an iron chelator, inhibited germ tube formation and growth of the dermatophyte *Trichophyton mentagrophytes* in microculture assays, and it was postulated that these results were due to iron deprivation.

Recent reports by Artis (1984) have presented evidence that the dermatophytic fungus *Trichophyton mentagrophytes* produces hydroxamate siderophores when cultured in a chemically defined, nonphysiologic low-iron medium. However, the exact chemical nature of the substances was not reported.

In spite of the probable key role that iron-transport chemistry plays in host invasion by pathogenic dermatophytes, there have been no reports in which iron-chelating agents have been identified within this important class of fungi. In this paper, we report the isolation and characterization of the principal siderophore produced by the dermatophyte *Microsporum gypseum* grown under iron-limiting conditions.

Materials and Methods

Microsporum gypseum Culture. (A) Preparation of Inoculum Medium. The inoculum medium was a modification of that of Wiebe and Winkelmann (1975) with the following composition: L-asparagine (5 g), $K_2HPO_4 \cdot 7H_2O$ (1 g), glucose (20% w/v, 100 mL), distilled, deionized water (900 mL). The pH of medium was adjusted to 6.0 prior to autoclaving at 121

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