Synthesis and Structure–Activity Study of Myxoma Virus Growth Factor†

Yao-Zhong Lin, Xiao-Hong Ke, and James P. Tam*
The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399
Received November 26, 1990; Revised Manuscript Received January 7, 1991

ABSTRACT: Myxoma virus growth factor (MGF) is an 85-residue peptide derived from the gene product of a DNA tumor virus that infects rabbits. The carboxyl domain of MGF possesses about 40% sequence homology with the epidermal growth factor (EGF). This EGF-like domain covering residues 30–83 was synthesized and found to possess putative activities of EGF. It was, however, about 200-fold less active than EGF in the competitive binding of human EGF receptor in A431 cells and the stimulation of [^{3}H]-thymidine uptake in NRK 49F cells. MGF(30–83) is a basic and a hydrophobic peptide rich in β-sheet structure. These features in MGF tend to promote aggregation, leading to precipitation even in strongly denaturing solutions. Thus, the refolding of MGF was achieved with difficulty and resulted in low yield. To increase the synthetic yield of MGF(30–83), a cluster of acidic amino acids was added to the NH_2-terminus of MGF(30–83). This approach was found to be effective in minimizing the refolding difficulties and allowed accessibility to the synthesis of analogues in this class of compounds. The relationships of structure and function of MGF were studied by using analogues with point substitution by the corresponding D-amino acid or by Ala at position 44 or 52 and analogues with deletion of basic residues from the amino terminus. Modifications of both the receptor contact and the structural residues greatly reduced the potency of MGF(30–83), and the overall result correlated well with the known structure–activity of the EGF family.

Myxoma virus, an agent of myxomatosis and a DNA tumor virus of the poxvirus family, was first found in South American rabbits. The myxoma virus was related to the Shope fibroma virus (Shope, 1932). However, myxoma virus has a broader host range than the Shope fibroma virus. It infects several types of mucosal cells, lymphocytes, and fibroblasts, whereas the Shope fibroma virus only infects fibroblasts (Strayer et al., 1983; Strayer & Sell, 1983).

More recently, this tumorigenic DNA virus was mapped and sequenced (Upton et al., 1987). It revealed an 85-residue gene product, myxoma virus growth factor (MGF).[1] MGF shares considerable sequence homology with epidermal growth factor (EGF) (Cohen, 1962) and transforming growth factor α (TGFα) (DeLarco & Todaro, 1978). The homologous region of MGF based on alignment of the conserved positions of six cysteinyl residues lies at the carboxyl portion of MGF (residues 30–83) and shares 42% amino acid homology with mouse EGF (mEGF) and rat TGFα (Upton et al., 1987). MGF also shares a high sequence homology with two other members of the DNA poxvirus family, Shope fibroma growth factor (SFGF) (Chang et al., 1987) and vaccinia growth factor (VGF) (Blomquist et al., 1984; Brown et al., 1985; Stroobant et al., 1985; Twardzik et al., 1985).

Despite the close sequence homology of MGF with the EGF family, it has not been determined whether MGF contains the putative EGF-like activities. To answer this question, we have synthesized a 54-residue MGF (residue 30–83) comprising the homologous region of the EGF/TGFα family (Figure 1). MGF(30–83) consists of a tricyclic and three-loop subdomains characteristic of the TGFα/EGF family and would consist of a structure similar to both rat and human TGFα. However, MGF(30–83) is 4 residues longer than TGFα in the second disulfide subdomain (residues 45–65), which contains 21 residues as compared to 17 residues found in TGFα.

The solution structures of EGFs (Montelione et al., 1986; Cooke et al., 1987) and TGFα (Brown et al., 1989; Kohda et al., 1989) based on ^{1}H NMR have been determined and are rich in reverse turns and β-sheets. Since MGF is a member of the EGF family, it is anticipated that MGF would contain similar structural features. However, unlike EGF and TGFα which are acidic, MGF(30–83) is basic and hydrophobic. It has a net of 3 basic charges, a combination of 12 Thr and Asn, and 23 hydrophobic amino acids. A major difficulty encountered in the synthesis of molecules rich in β-sheet and β-branched residues is aggregation and precipitation in neutral and basic media. Such difficulties in the refolding process after

Table I: Sequences of Synthetic Myxoma Virus Growth Factor and Analogues[a]

Peptides	Sequences
A:	I I K R I K V C N D D Y K N Y C L N N G T C F T V
B:	- - - - - - - - - - Y* - - - - - - - - -
C:	- - - - - - - - - - A - - - - - - - - -
D:	- - - - - - - - - - - - - - - - - - F* - -
E:	D E E E -
F:	- - - - - - S - - -
A:	A L N N V S L N P F C A C H I N Y V G S R C Q F I N L I T
B:	- -
C:	- -
D:	- -
E:	- -
F:	- -

[a] Peptide A, MGF(30–83); B, [D-Tyr44]-MGF(34–83); C, [Ala44]-MGF(34–83); D, [D-Phe52]-MGF(34–83); E, [Asp26,Glu27,Glu28,Glu29]-MGF(26–83); F, [Ser51]-MGF(45–83). The residues with asterisks are D-amino acids.

† This work was supported by USPHS Grant CA 36544, awarded by the National Cancer Institute, DHHA.
* To whom correspondence should be addressed.

[1] Abbreviations: Boc, *tert*-butoxycarbonyl; EGF, epidermal growth factor; HPLC, high-performance liquid chromatography; MGF, myxoma virus growth factor; SFGF, Shope fibroma virus growth factor; TFA, trifluoroacetic acid; TGFα, transforming growth factor type α; VGF, vaccinia virus growth factor.

0006-2960/91/0430-3310$02.50/0

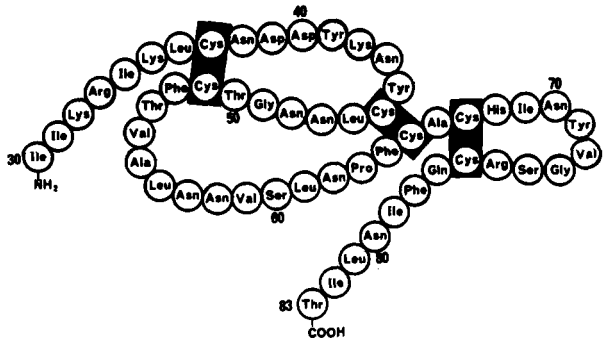

FIGURE 1: Structure of MGF(30–83).

the chemical synthesis were also encountered with the basic and hydrophobic SFGF, a molecule with 80% sequence homolgy with MGF (Lin et al., 1988). Reduced SFGF precipitated from the 5 M urea solution in pH 8.0–8.5. This difficulty of refolding is the major contributing reason to the low synthetic yield of SFGF. To alleviate this problem, we devised two approaches in the design of analogues consisting of deleted sequences and point-substituted analogues on the conserved residues (Table I). Both approaches are aimed to decrease the basicity of MGF analogues. Because the NH_2-terminus is not important for biological activity, we deleted the amino-terminal tetrapeptide containing two basic residues (30–33, Ile-Ile-Lys-Arg) in one of the approaches (analogues B–D; see Table I). In another approach (analogue E, Table I), a cluster of acidic amino acids is added at the NH_2-terminus of MGF to give analogue E an overall acidic charge. These approaches are designed with the consideration to increase synthetic yield without greatly affecting the biological activity of MGF.

In addition, analogues are designed to test the importance of the first and second subdomains of MGF. Tyr-44 in the first subdomain is highly conserved in the TGFα/EGF family, and Phe-52 in the second subdomain is conserved in the SFGF. To examine the importance of the aromatic moiety in its role to form aromatic clusters to stabilize its structure, Tyr-44 is substituted with either Ala (analogue C) or D-Tyr (analogue B). Similarly, Phe-52 is also substituted with D-Phe (analogue D) to test the importance of β-sheet function in the backbone structure. The D-amino acid is expected to disrupt the antiparallel β-sheet formation. Finally, the first subdomain of MGF is deleted in analogue F to determine its importance toward biological activity.

Experimental Procedures

Syntheses of MGF(30–83) and Analogues. MGF(30–83) (peptide A) and analogues including [D-Tyr-44]-MGF(34–83) (peptide B), [Ala-44]-MGF(34–83) (peptide C), [D-Phe-52]-MGF(34–83) (peptide D), [Asp-26, Glu-27, Glu-28, Glu-29]-MGF(26–83) (peptide E), and [Ser-51]-MGF(45–83) (peptide F) (Table I) were carried out manually on N^{α}-*tert*-Boc-Thr(BrZ)-OCH_2-Pam resin (Mitchell et al., 1978; Tam et al., 1979). All amino acids were protected with the N^{α}-*tert*-butoxycarbonyl (Boc) group. The side-chain protections were as follows: Arg(Tos), Asp(OBzl), Cys(4-MeBzl), Glu(OBzl), His(Dnp), Lys(2-ClZ), Ser(Bzl), Thr(Bzl), and Tyr(2-BrZ). Each synthetic cycle consisted of (i) a 20-min deprotection by trifluoroacetic acid (TFA/CH_2Cl_2, 1:1 v/v) preceded by two TFA prewashes containing 0.05% each of ethanedithiol and dimethyl sulfide (DMS) for 1 min each, (ii) neutralization with 5% diisopropylethylamine/45% CH_2Cl_2/50% dimethylformamide (DMF), and (iii) double coupling with 3 equiv each of Boc-amino acid and dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 for 45 min, and then in CH_2Cl_2/DMF (1:1, v/v) for 15 min (Tam et al., 1986; Lin et al., 1988). A third coupling of symmetrical anhydride was used when necessary. Double couplings of Boc-Asn, Boc-Gln, and Boc-Arg(Tos) were performed by the hydroxybenzotriazole (HOBt) active esters in DMF. The yield in each coupling was monitored by a quantitative ninhydrin test (Sarin et al., 1981). The cumulative yields were 90–95% on the basis of back-hydrolysis of the peptide resins.

Each protected peptide resin (0.5 g) were treated 3 times with 10 mL of a 1 M solution of thiophenol in DMF for 8 h to remove the N^{im}-Dnp protecting group of His and then with 50% TFA/CH_2Cl_2 (v/v, 10 mL) for 20 min to remove to N^{α}-Boc group. The dried peptide resin was premixed with DMS, *p*-cresol, *p*-thiocresol, and 100 mg of cysteine. HF was transferred into the mixture at −78 °C to reach a final volume of 10 mL (HF/DMS/*p*-cresol/*p*-thiocresol, 25:65:7.5:2.5, v/v). After the reaction was stirred at 0 °C for 2 h, HF and DMS were removed in vacuo. The residue was recharged with liquid HF at −78 °C to reach a total volume of 15 mL. The reaction was performed at 0 °C for 1 h (Tam et al., 1983). After the HF was removed, the resulting residue was washed twice with 80 mL of 2% mercaptoethanol solution in ether and then extracted with 100 mL of 8 M urea/0.2 M dithiothreitol (DTT)/0.1 M Tris-HCl buffer, pH 8.2. The cleavage yields were about 90% based on the back-hydrolysis of the HF-treated resin by propionic acid/12 N HCl (1:1, v/v). The peptide solutions were dialyzed sequently (Spectrum 6, molecular weight cutoff 1000) against 2 L each of deoxygenated and N_2-purged 8, 5, and 2 M urea/0.1 M Tris-HCl buffer, pH 8.2, and then oxidized in the presence of reduced and oxidized glutathiones (1 mmol each) with occasional stirring for 36–96 h (Lin et al., 1988; Saxena & Wetlaufer, 1970). The solutions containing the crude peptide mixtures were purified by preparative reverse-phase C_{18} liquid chromatography (2.5 × 30 cm) eluted with 0.045% TFA/aqueous acetonitrile at a flow rate of 20 mL/min. The major fractions were lyophilized and then further purified on a second C_{18} reverse-phase liquid chromatography eluted with 0.045% TFA/acetonitrile at a shallower gradient system. The total synthetic yields ranged from 1 to 10%, based on the first amino acid loading to the resin.

Characterizations of MGF(30–83) and Analogues. Amino acid analysis of each synthetic peptide was carried out in 5.7 N HCl for 24 h at 110 °C and was found to agree with the theoretical ratio. Analytical HPLC of a 5-μg sample was performed on a Vydac column (C_{18} reverse phase) with a 30-min linear gradient of 15–85% buffer B at a flow rate of 1.5 mL/min (Figure 2) (buffer A, 5% CH_3CN, 95% H_2O, and 0.045% TFA; buffer B, 60% CH_3CN, 40% H_2O, and 0.039% TFA). The synthetic peptides were also analyzed by Cf-252 fission ionization mass spectrometry (Table II) (Chait et al., 1982; Macfarlane & Torgerson, 1976).

Biological Assays. After 1-h incubations at 22 °C with synthetic MGF(30–83) and analogues, inhibition of ^{125}I-EGF binding to the EGF receptor was examined on subconfluent monolayers of formalin-fixed A-431 cells (Table III) (DeLarco & Todaro, 1978). The [^{3}H]thymidine assays were carried out on normal rat kidney fibroblasts, clone 49 F in DMEM with 10% heat-inactivated calf serum for 24 h at 37 °C, and maintained in medium containing 0.2% calf serum for 3 days. Incorporation of [^{3}H]thymidine was counted after 24-h exposure (Table III) (DeLarco et al., 1981).

Results

Synthesis and Characterization. The conventional stepwise solid-phase approach (Merrifield, 1963, 1986) was adopted

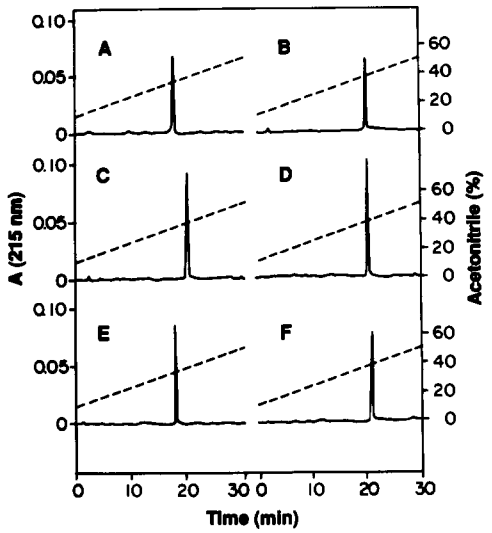

FIGURE 2: Analytical C_{18} reverse-phase HPLC-purified synthetic MGF(30–83) and its analogues (structures A–F; see Table I).

Table II: Cf-252 Fission Fragment Mass Spectrometry of MGF(30–83) and Analogues

peptides[a]	MW from $(M + H)^+$	MW from $(M + 2H)^{2+}$	calcd values	Δ[b]
A	6154.4	6154.6	6154.2	+0.3
B	5643.8	5643.2	5643.4	+0.1
C	5550.9	5550.6	5551.4	−0.6
D	5643.6	5643.4	5643.4	+0.1
E	6656.8	6656.6	6656.6	+0.1
F	4259.2	4258.8	4258.8	+0.2

[a] Peptide A, MGF(30–83); B, [D-Y44]-MGF(34–83); C, [A44]-MGF(34–83); D, [D-F52]-MGF(34–83); E, [D26,E27,-28,-29]MGF-(26–83); F, [S51]-MGF(45–83). [b] Δ is the difference between calculated and measured masses.

Table III: Comparison of Synthetic MGF Analogues by Different Assays

peptides	binding A431 IC_{50} (μM)[a]	relative to MGF	thymidine assay EC_{50} (μM)	relative to MGF
A	0.4	1	0.07	1
B	24	0.02	0.35	0.20
C	7	0.06	1.50	0.05
D	53	0.01	0.50	0.14
E	0.4	1	0.70	0.10
F	27	0.02		

[a] IC_{50} inhibition of binding of 0.7 nM ^{125}I-EGF to A431 cells. [b] Incorporation of [^{3}H]thymidine was measured in NRK 49F after 8 h.

for the syntheses of the MGF(30–83) and its analogues. We used a differential acid-labile protecting group scheme with a TFA-labile *tert*-butoxycarbonyl group for the NH_2-terminus and HF-labile benzyl alcohol derived groups for the side-chain protection. A more acid-stable linkage to the solid support, Pam resin, was used for the synthesis to reduce the loss of peptide chain during the repetitive acid–deprotection cycles (Mitchell et al., 1978; Tam et al., 1979). The double coupling protocol with DCC was used except in Boc-Arg, Boc-Asn, and Boc-Gln, and quantitative coupling yields were obtained in nearly all cycles. A more polar cosolvent mixture of CH_2Cl_2 and DMF was used instead of CH_2Cl_2 alone at the second stage of the coupling steps to fully solvate the peptide resins and to facilitate the coupling reaction (Lin et al., 1988; Tam, 1987). However, complete couplings in Asn-47 and Gln-77 were difficult to achieve even after triple couplings. The remaining 2% of free amino groups were blocked by acetic anhydride. Interestingly, similar difficulties also occurred in the synthesis of SFGF(26–80) (Lin et al., 1988). In the repetitive TFA deprotecting steps, small amounts of ethanedithiol and DMS (0.05% each by volume) were added into the TFA prewash solution to scavenge the *tert*-butyl cation and to protect possible sulfonium salt formation of thiol ether groups on the peptide chains. The low–high HF procedure (Tam et al., 1983) was used for the deprotection of side-chains and cleavage of the peptide chain from the resin, to iminimize the danger of the alkylation and acylation side reactions.

To refold the reduced peptide which was extracted from the resin in 8 M unit solution, it was treated in a continuous gradient against lower concentrations of urea (8–2 M). At the 2 M urea stage, the thiols were oxidized into disulfides in a gradient of increasing oxidizing potential in a mixture containing reduced and oxidized glutathiones. Following this general procedure, we found that MGF(30–80) aggregated extensively and precipitated from the solution at the 2 M urea stage. Disulfide oxidation had to be performed at the 6 M urea stage. The aggregation could be due to the strong basicity of MGF. Reduction of basicity of MGF might lead to reduction of aggregation. Thus, analogues B–D (Table I) were synthesized with four residues shorter than MGF(30–83) at the NH_2-terminus so that the basic residues, Lys-32 and Arg-33, were eliminated. Deletion of the basic tetrapeptide Ile-Ile-Lys-Arg improved the situation, and folding could be conducted at the 2 M urea stage but was not sufficient to avoid altogether the aggregation and precipitation. [Asp-26, Glu-27, Glu-28, Glu-29]-MGF(26–83) was designed by adding four acidic residues to the NH_2-terminus (Table I, peptide E). As expected, this peptide had much better solubility in the urea solution, and only very slight precipitation was found during the refolding and oxidation process. This improvement led to a higher refolding yield which was about 3–10-fold higher than in the parent MGF(30–83) and other analogues. The duration for the oxidation reaction depended upon the nature of the peptides and ranged from 2 to 48 h as monitored by C_{18} reverse-phase HPLC. The reaction mixtures after oxidation were purified to homogeneity by two or three successive C_{18} reverse-phase liquid chromatography stages in 1–10% overall yields. The low yields could be attributed to aggregation and precipitation during the refolding process. Amino acid analyses of MGF(30–83) and its analogues agreed well with the expected compositions. The molecular masses of all the purified peptides were determined by Cf-252 fission ionization mass spectrometry. In each case, peaks corresponding to $(M + H)^+$ and $(M + 2H)^{2+}$ were found, and they agreed well with the calculated values (Table II). The correct mass obtained from the mass spectrometry also confirmed that these synthetic peptides had been oxidized and folded to form three disulfide loops. Unoxidized forms containing free sulfhydryls would give 2–6 additional mass units in the $(M + H)^+$ ion. Mass spectrometry also eliminated the possibility of the formation of oligomers since no other peaks corresponding to these products were detected. Analytical high-performance liquid chromatography (HPLC) showed that each purified MGF analogue was eluted as a single symmetrical peak (Figure 2). Interestingly, the deletion of the tetrapeptide containing four basic residue resulted in longer retention time in the reverse-phase HPLC. However, analogue E with four acidic residues at the NH_2-terminus had a comparable elution time as its parent compound (analogue A).

Biological Activity. The 54-residue MGF(30–83) was found to compete with ^{125}I-EGF in the EGF receptor binding assay using a human epidermal carcinoma A431 cell with an IC_{50} of 0.4 μM and was about 0.5 and 3%, respectively, as active as murine EGF and SFGF(26–80) (Table III). [D-Tyr-

44]-MGF(34–83) (peptide B) and [Ala-44]-MGF(34–83) (peptide C), which contained replacement of the highly conserved Tyr-44 at the first disulfide subdomain, were about 2 and 6% as active as the parent MGF(30–83). Another point-substituted analogue, [D-Phe-52]-MGF(34–83)(peptide D), with replacement of Phe-52 at the second disulfide domain, was 1% as active as MGF(30–83) and found to have an IC_{50} of 53 μM. [Ser-51]-MGF(45–83) (peptide F), with deletion of the first disulfide domain of MGF, was about 2% as active as MGF(30–83). However, [Asp-26, Glu-27, Glu-28, Glu-29]-MGF(26–83), the analogue with an addition of four acidic residues at the NH_2-terminus of MGF(30–83), possessed similar EGF receptor binding activity to MGF(30–83). In the mitogenic assay (Table III), synthetic MGF(30–83) was found to stimulate [^{3}H]thymidine uptake in normal rat kidney fibroblasts with an EC_{50} at 0.07 μM. As expected, correlation was found between the receptor binding and mitogenic activities in analogues B–D which were 5–20% as active as the parent MGF(30–83). Interestingly, analogue E, with a cluster of acidic residues at the NH_2-terminus, showed slight dissociation between the mitogenicity and binding activity. While it was as active as MGF(30–83) in receptor binding, it was about 10-fold less active in the mitogenic assay.

Discussion

The results of this paper could be grouped into three categories, and all are related to MGF. First, our results show that MGF contains the putative biological activity of EGF. Second, our results present problems and solutions associated with the synthesis of β-sheet-rich peptides such as MGF. Finally, we explore the structure and activity relationships of MGF and its pertinence to the EGF family.

On the basis of sequence homology, the EGF-like domains of the poxvirus family including VGF, SFGF, and MGF are predicted to contain putative EGF activities. VGF from vaccinia virus has been shown to compete with EGF for receptor binding in the A431 cell but exhibits different levels of mitogenic activity in other cell lines (Lin et al., 1990). VGF shows low levels of mitogenic and colonogenic activities in NRK clone 49F cells and is an antagonist in these cell lines. However, VGF shows full agonist activities to Swiss 3T3 cells and partial agonist activities in NRK clone 3 cells. SFGF of Shope fibroma virus has been synthesized and shown to be an active EGF-like mitogen both in vitro and in vivo (Lin et al., 1988; Ye et al., 1988). Our results show that MGF, the third member of the DNA tumor virus, is also an active member of the EGF family.

The synthetic EGF-like domain of myoxma growth factor binds to the EGF receptor and is mitogenic to NRK 49F cells. However, in comparison with EGF, TGFα, or other known poxvirus-encoded EGF-like protein products, MGF(30–83) has the lowest biological potency. It is about 100–200-fold lower in biological potency than EGF/TGFα and 30–80-fold lower than SFGF and VGF. The role of the EGF-like growth factor of the poxvirus family in the host pathogenesis of the DNA tumor virus remains obscure. The presence of EGF/TGFα-like growth factors in tumorigenic poxviruses such as Shope fibroma and myxoma viruses has led to the hypothesis that these gene products may affect growth and progression of poxvirus-infected cells and may play an important role in the life cycle of the poxvirus.

The viability of chemical synthesis of this family of viral growth factors depends largely on an efficient method from refolding and oxidation of synthetic material to the correct product. Since MGF(30–83) is a very basic and hydrophobic peptide, it has a strong tendency of aggregation and precipitation during the refolding process, resulting in low synthetic yields (1–2%). An acidic analogue, [Asp-26,Glu-27,Glu-28,Glu-29]-MGF(26–83), with four acidic residues added on the NH_2-terminus was designed and shown to possess better solubility in the refolding process to give a 10-fold higher synthetic yield than the parent compound. Furthermore, this is achieved without greatly affecting the biological potency of MGF(30–83). Thus, the approach of adding acidic amino acid clusters to overcome the refolding problems of basic and hydrophobic peptides and proteins may be generally useful for similar proteins.

A question raised from our results is the apparent lower affinity of MGF to the EGF receptor on a human cell line. There are two plausible explanations. First, the rabbit EGF receptor may be structurally different from the human EGF receptor. Since human EGF receptor is being used for the receptor assay, the difference in MGF binding affinity to the EGF receptors may account for the lower affinity observed. Second, the role of the amino-terminal 29-residue peptide (1–29) in MGF has yet to be determined. This peptide may assist in stabilizing the carboxyl EGF-like domain and hence in the receptor binding affinity.

Another question is whether MGF binds to the same site as EGF on the EGF receptor. We propose to answer this question indirectly by testing a series of analogues. If the same site is involved, there will be a strong correlation between the structure–activity relationship of EGF and MGF. In the first analogue, we test Tyr-44 which is a highly conserved aromatic residue of the first subdomain in the EGF/TGFα family. In all known active sequences, this position is occupied by either Tyr or Phe. This aromatic residue is believed to be a receptor contact residue. It was reported that replacement of Phe by Ala in human TGFα (Defeo-Jones et al., 1988) leads to substantial reduction of biological activity. [D-Tyr-44]-MGF(34–83) and [Ala-44]-MGF(34–83) exhibit about 20–50-fold lower potency in the receptor binding assay in comparison to the parent MGF(30–83). This result is consistent with the finding that residue Tyr-44 is important to the EGF receptor binding activity. In contrast, Phe-52 is not conserved in the EGF family. [D-Phe-52]-MGF(34–83) at the second subdomain exhibits about 100-fold lower activity in the EGF receptor binding assay in comparison to MGF(30–83). Phe-52 structurally is involved in a double-stranded antiparallel β-sheet structure that acts as a scaffold for receptor contact residues and does not contribute directly to the receptor binding site. In fact, the sizes of the β-sheet regions are not conserved in the EGF-like growth factors. In MGF and SFGF, this major β-sheet is extended three amino acid residues longer than EGF and four residues longer than TGFα. Nonetheless, the D-Phe-52 analogue distorts β-sheet formation and the overall structure of the receptor contact region. Analogue E, with an extended amino terminus, exhibits the same binding activity to the EGF receptor as to the parent MGF(30–83). This finding is consistent with others that the NH_2-terminal region of EGF or TGFα is not important for receptor binding (Lin et al., 1990). However, complete removal of the first subdomain which includes the invariant Tyr-44 in [Ser-51]-MGF-(45–83) greatly reduces receptor binding activity. Overall, results obtained from the structure–activity study of MGF are consistent with those obtained from EGF and TGFα (Tam et al., 1989, 1990a,b). Thus, we can conclude that it is likely that MGF and EGF compete at the same site as the EGF receptor but at a lower affinity.

Acknowledgments

We thank Drs. B. T. Chait and F. H. Field of The Rock-

efeller University Mass Spectrometry Biotechnology Resource and D. G. Kolodin for his technical assistance.

Registry No. EGF, 62229-50-9.

REFERENCES

Block, W., Upton, C., & McFadden, G. (1985) *Virology 140*, 113–124.

Blomquist, M. C., Hunt, L. T., & Barker, W. C. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7363–7367.

Brown, J. P., Twardzik, D. R., Marquardt, H., & Todaro, G. J. (1985) *Nature (London) 313*, 491–492.

Brown, S. C., Mueller, L., & Jeffs, P. W. (1989) *Biochemistry 28*, 593–599.

Carpenter, G., & Cohen, S. (1979) *Annu. Rev. Biochem. 48*, 193–216.

Chait, B. T., Gisin, B. F., & Field, F. H. (1982) *J. Am. Chem. Soc. 104*, 5157–5162.

Chang, W., Upton, C., Hu, S. L., Purchio, A. F., & McFadden, G. (1987) *Mol. Cell. Biol. 7*, 535–540.

Cohen, S. (1962) *J. Biol. Chem. 237*, 1555–1562.

Cooke, R. M., Wilkinson, A. J., Baron, M., Pastore, A., Tappin, M. J., Campbell, I. D., Gregory, H., & Sheard, B. (1987) *Nature (London) 327*, 339–341.

Defeo-Jones, D., Tai, J., Wegrzyn, R. J., Vuocolo, G. A., Baker, A. E., Payne, L. S., Garsky, V. M., Oliff, A., & Riemen, M. W. (1988) *Mol. Cell. Biol. 8*, 2999–3007.

De Larco, J. E., & Todaro, G. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4001–4005.

De Larco, J. E., Preston, Y. A., & Todaro, G. J. (1981) *J. Cell. Physiol. 109*, 143–152.

Kohda, D., Shimada, I., Miyake, T., Fuwa, T., & Inagaki, F. (1989) *Biochemistry 28*, 953–958.

Lin, Y. Z., Caporaso, G., Chang, P. Y., Ke, X. H., & Tam, J. P. (1988) *Biochemistry 27*, 5640–5645.

Lin, Y. Z., Ke, X. H., & Tam, J. P. (1990) *J. Biol. Chem.* (in press).

Macfarlane, R. D., & Torgerson, D. F. (1976) *Science (Washington, D.C.) 191*, 920–925.

Merrifield, R. B. (1963) *J. Am. Chem. Soc. 85*, 2149–2154.

Merrifield, R. B. (1986) *Science (Washington, D.C.) 232*, 341–347.

Mitchell, A. R., Kent, S. B. H., Engelhard, M., & Merrifield, R. B. (1978) *J. Org. Chem. 43*, 2845–2852.

Montelione, G. T., Wuthrich, K., Nice, E. C., Burgess, A. W., & Scheraga, H. A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8594–8598.

Montelione, G. T., Wuthrich, K., Nice, E. C., Burgess, A. W., & Scheraga, H. A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5226–5230.

Sarin, V. K., Kent, S. B. H., Tam, J. P., & Merrifield, R. B. (1981) *Anal. Biochem. 117*, 147–157.

Saxena, V. P., & Wetlaufer, D. B. (1970) *Biochemistry 9*, 5015–5023.

Shope, R. E. (1932) *J. Exp. Med. 56*, 793–802, 803–822.

Strayer, D. S., & Sell, S. (1983) *JNCI, J. Natl. Cancer Inst. 71*, 105–111.

Strayer, D. S., & Leibowitz, J. L. (1987) *Am. J. Pathol. 128*, 203–209.

Strayer, D. S., Cabirac, G., Sell, S., & Leibowitz, J. L. (1983) *JNCI, J. Natl. Cancer Inst. 71*, 91–104.

Stroobant, P., Rice, A. P., Guillick, W. J., Cheng, D. J., Kerr, I. M., & Waterfield, M. D. (1985) *Cell 42*, 383–393.

Tam, J. P. (1987) *Int. J. Pept. Protein Res. 29*, 421–431.

Tam, J. P., Kent, S. B. H., Wong, T. W., & Merrifield (1979) *Synthesis*, 955–857.

Tam, J. P., Heath, W. F., & Merrifield, R. B. (1983) *J. Am. Chem. Soc. 105*, 6442–6455.

Tam, J. P., Sheikh, M. A., Salomon, D. S., & Ossowski, L. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8082–8086.

Tam, J. P., Lin, Y. Z., Wu, C. R., Shen, Z. Y., Galantino, M., Liu, W., & Ke, X. H. (1990a) in *Peptides: Chemistry, Structure and Biology; Proceedings of the Eleventh American Peptide Symposium*, p 75, ESCOM Science Publishers B. V., The Netherlands.

Tam, J. P., Lin, Y. Z., Shen, Z. Y., Ke, X. H., Wu, C. R., Galantino, M., & Liu, W. (1990b) *UCLA Symposia on Molecular & Cellular Biology, Synthetic Peptides: Approaches to Biological Problems*, Frisco, CO (in press).

Twardzik, D. R., Brown, J. P., Ranchalis, J. E., Todaro, G. J., & Moss, B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 5300–5304.

Upton, C., Macen, J. L., & McFadden, G. (1987) *J. Virol. 61*, 1271–1275.

Ye, Y. K., Lin, Y. Z., & Tam, J. P. (1988) *Biochem. Biophys. Res. Commun. 154*, 497–501.