Cloning and sequence analysis of a cDNA for rat epidermal growth factor

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Epidermal growth factor (EGF) is a mitogenic polypeptide capable of stimulating the proliferation of both mesenchymal and epithelial cells (1). Previously we have shown that EGF isolated from the submaxillary gland of the rat (rEGF), while missing the C-terminal 5 amino acid residues present in both mouse and human EGFs, is equipotent with mEGF in both receptor binding affinity to human A431 cells and mitogenic activity for mouse 3T3 cells (2). We have cloned a cDNA encoding the EGF precursor from an adult rat kidney λ gt 11 library (Clontech Laboratories Inc., Palo Alto, CA). The library was screened with a mEGF precursor cDNA probe (obtained from Dr. G.I. Bell, Chiron Co., Emeryville, CA) containing the EGF coding region (3). A number of clones were identified and the nucleotide sequence of one of these (3.3 kb) encoding mature rEGF was determined.

			5						-1			1			5						10		
Trp	Lys	Arg	Trp	Leu	His	Trp	Val	Trp	Asn	Ser	Asn	Thr	Gly	Cys	Pro	Pro	Ser	Tyr	Asp	Gly			
TGG	AAA	AGA	TGG	CTG	GAC	TGG	GTC	ATG	AAC	AGT	AAC	ACA	GGA	TGC	CCG	CCG	TCG	TAC	GAT	GGG			
			15			20						25			30								
Tyr	Cys	Leu	Ctc	Aan	Gly	Gly	Cys	Tgc	Tyr	Tat	Gtt	Gaa	Val	Asp	Gac	Tyr	Tac	Cys	Asn	Tgt			
TAC	TGC	CTC	AAT	GGT	GGC	GTG	ATG	TAT	TAT	GTT	GAA	TCC	GTG	AGC	CGC	TAC	GTG	TGC	AAC	TGT			
			35			40			45			50			55								
Val	Gly	Ile	Tyr	Ile	Gly	Glu	Cys	Gln	His	Arg	Asp	Leu	Arg	Trp	Asn	Trp	Arg	His					
GTC	ATT	GGC	TAT	ATT	GGA	GAA	TGT	CAG	CAC	GGA	GAC	TTA	CGT	TGG	TGG	TGG	CGC	CAT					
			60																				
Ala	Asp	Tyr	Gly	Gln	Arg	His	Asp	Ile	Thr	Val	Val	Ser	TCT	GTG	TGT	Val	Ala	Leu	Glu				
GCT	GAC	TAC	GGG	CAG	AGG	CAC	GAC	ATC	ACT	GTG	GTG	TCT	GTG	TGT	GTG	GTG	GCG	CTG	CTG				
			80						85						90			95					
Leu	Leu	Leu	Leu	Met	Trp	Gly	Gly	Thr	Tyr	Tyr	Tyr	Arg	Thr	Arg	Lys	Gln	Leu	Ser	Glu				
CTG	CTC	CTC	CTC	GGG	ATG	TGG	GGG	ACT	TAC	TAC	TAC	AGG	ACT	CGG	AAG	CAG	CTA	TCA	GAG				
			100						105			110			115			120					
Ser	Lys	Lys	Pro	Ser	Glu	Glu	Ser	Ser	Ser	Asn	Val	Ser	Ser	Asn	Gly	Pro	Asp	Ser	Ser				
TCA	AAG	AAG	CCT	TCC	GAA	GAG	TCA	AGC	AGC	AAC	GTG	AGC	AGT	AAC	GGG	CCT	GAC	AGC	AGC				
			120			125						130			135								
Ala	Gly	Val	Ser	Ser	Gly	Pro	Gln	Pro	Trp	Phe	Val	Val	Gln	Glu	Glu	His	Gln						
GCT	GGG	GTG	TCT	TCT	GGT	CCC	CAA	CCT	TGG	TTT	GTG	GTC	CTA	GAG	GAA	CAC	CAA						

The cDNA-deduced amino acid sequence of rEGF, delineated by the heavy underlining, is in perfect agreement with the protein sequence determined by Edman degradation (2). Interestingly, the C-terminal pentapeptide missing in the isolated rEGF (2), but found in isolated murine (1) and human (4) EGFs, is present in the rEGF precursor (residues 49-53, underlined with dots, Trp-Trp-Asn-Trp-Arg). The corresponding residues in mouse and human EGF are Trp-Trp-Glu-Leu-Arg. It is clear from recent studies by Burgess *et al.* (5) that the last 5 residues of mEGF do not influence either the receptor binding, *in vitro* mitogenic activity or the ability of the reduced molecule to refold into the biologically active form. Human EGF₁₋₄₇ is reported to be equipotent with the native protein *in vivo* (6). Since rEGF was isolated from submaxillary glands (2) using the same acidic extraction conditions employed for mEGF (1% CF₃COOH/5% HCOOH) (7), the removal of the C-terminal pentapeptide of rEGF (residues 49-53) is due, presumably, to differential processing in the submaxillary gland. One possible explanation is that the alteration of two amino acids in rEGF precursor near the site of C-terminal processing (residues in m- and hEGF are Glu-Leu and in rEGF Asn-Trp), may affect the substrate specificity of a putative arginine esterase. Alternatively, there may be a very potent arginine esterase present in crude rat (but not mouse) submaxillary gland homogenates.

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