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Abnormal Products of the Human Insulin Gene

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SUMMARY

Heritable abnormalities in the insulin gene have often been considered in terms of their potential for contributing to diabetes. It is only within the last 5 yr, however, that evidence has demonstrated the existence of insulin gene mutations in man and the secretion of abnormal human insulins in affected individuals. HPLC analysis of insulin purified from serum by immunoaffinity chromatography and detection of insulin by radioimmunoassay have documented abnormal insulins in subjects from three separate families. HPLC analysis of these natural insulins and of semisynthetic insulin analogues have identified the accompanying amino acid substitutions in two of these cases: in one, leucine replaces phenylalanine B25; in the other, serine replaces phenylalanine B24. Both substitutions occur in an invariant tetrapeptide sequence within the active site of the hormone. Studies of the biologic activities of these analogues further suggest that replacements at position B25 result in the loss of an important side chain contact between the hormone and its receptor, whereas those at position B24 result in conformational changes in the insulin molecule as a whole. Two additional individuals have been identified to secrete abnormal intermediates of proinsulin conversion in which the C-peptide remains attached to the insulin A-chain and in which Arg^{65} has been replaced by a nonbasic amino acid. This result emphasizes the importance of dibasic amino acid pairs at prohormone conversion sites and provides clues about the evolution of hormone precursors. Thus, studies of the products of abnormal human insulin genes have provided insights into subjects as varied as insulin biosynthesis, structure-activity relationships in insulin recognition by receptors, and the physiology of insulin action. DIABETES 33:693–699, July 1984.

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Although diabetes is a multifaceted disease with several causes and with complex consequences, any consideration of the disease must deal with the importance of B-cell function and the production of adequate amounts of active insulin. The discovery of proinsulin, the single-chained biosynthetic precursor to insulin, by Steiner and his colleagues in 1967, explained how a hormone with the structure of insulin could be synthesized by ordinary cellular processes and how the two-chained hormone could be formed by limited proteolytic events that convert the precursor to its final product.[1-3] In the same year, Kimmel and Pollack suggested that alterations in the structure of insulin might in part account for the abnormal glucose regulation seen in some diabetic patients.[4] These two separate observations, together with much more recent information on the structure of the human insulin gene,[5,6] have led the way to detailed considerations of insulin gene mutations in man, abnormal products of the human insulin gene, and the impact of abnormal insulins on human physiology.

Our knowledge of processes that are required for insulin biosynthesis in the pancreatic B-cell has expanded greatly in recent years. The biosynthesis of this life-sustaining hormone requires (1) transcription of the insulin gene, (2) maturation of the insulin mRNA precursor, (3) translation of the mature mRNA, (4) translocation of the product (preproinsulin) to the cisternum of the endoplasmic reticulum with removal of the NH_2-terminal signal sequence, (5) folding of the newly synthesized proinsulin, (6) packaging of the precursor into secretion granules, and (7) conversion of the single-chained precursor to two-chained insulin.[5-9] The complexity of these processes suggests the need for great precision in the many steps that lead to the formation of insulin. In fact, the number of steps involved in the biosynthesis of insulin greatly exceeds the number involved in the biosynthesis of cytosolic enzymes or other proteins where mutation is well known to cause what we call inborn errors of metabolism.

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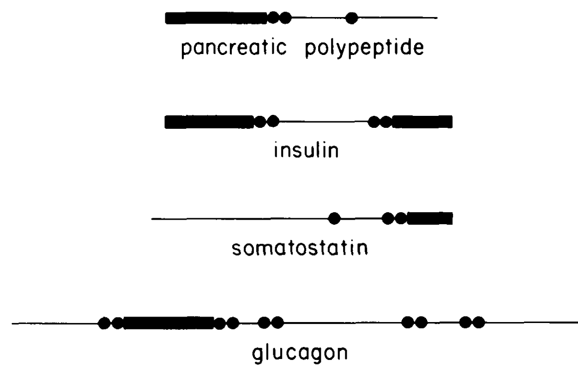

FIGURE 1. Diagrammatic structures of the precursors for the four major endocrine cell products of the pancreatic islet. Portions of the precursors that correspond to the named hormones are indicated by heavy bars, whereas portions that correspond to peptide extensions are indicated by lines; dibasic amino acid residues (arginine or lysine) corresponding to precursor conversion sites are shown as filled circles. Note that proinsulin has been drawn in an extended form that does not show disulfide bonds; the structure of proinsulin illustrated has the sequence B-chain–C-peptide–A-chain.

Identification of individuals who might secrete abnormal products of the human insulin gene requires very detailed clinical investigation. Rubenstein and his co-workers have carefully categorized the characteristics of individuals who secrete abnormal insulin gene products;[10-12] these characteristics include hyperinsulinemia without hypoglycemia, no evidence for insulin resistance due to either blood-borne factors or decreased numbers of insulin receptors, reduced biologic activity of circulating insulin, and normal response to exogenous insulin. The first and last of these findings are perhaps the most important: if a subject maintains higher than normal levels of circulating insulin without hypoglycemia, but responds as expected to the administration of exogenous insulin, it must be concluded that the individual's immunoreactive serum insulin is somehow faulty and that it is structurally different from normal insulin. In the broadest sense, such abnormal products of the human insulin gene may be closely related to the hormone precursor (molecular weight about 9000) or to the hormone itself (molecular weight about 6000). In all, individuals from five different families have now been identified as secreting abnormal insulin gene products. Subjects from three of these families secrete abnormal insulins; subjects from the other two secrete abnormal intermediates of proinsulin conversion. In each case, study of these abnormal peptides has provided important information on the mechanisms of insulin biogenesis and action.

METHODS

Methods used in detecting and identifying the abnormal products of the human insulin gene described in this article, including those for insulin purification from pancreas[13] and serum[10,14,15] for the chemical modification of insulin-related peptides by oxidative sulfitolysis and acetylation,[14,16] for the HPLC analysis of peptides isolated from serum,[12,15-17] and for the semisynthesis of insulin analogues[17,18] have been described before. These citations give additional references for detailed analytic procedures.

RESULTS AND DISCUSSION

Hormone precursor structure. The biosynthesis of peptide hormones of the pancreatic islet, like the biosynthesis of a great many peptide effectors of metabolism, requires the posttranslational, enzymatic processing of higher-molecular-weight hormone precursors. In each case, endoproteolytic events catalyzed by trypsin-like enzymes occur at sites containing paired dibasic amino acid residues; in some cases these endoproteolytic events are followed by exoproteolytic events catalyzed by carboxypeptidase B-like enzymes that remove COOH-terminal dibasic amino acid residues.[7,9,19] As illustrated in Figure 1 for the hormones of the pancreatic islet, the hormone sequence may occur at the NH_2-terminus of the precursor (as for pancreatic polypeptide[20,21]), at the COOH-terminus of the precursor (as for somatostatin[22,23]), at

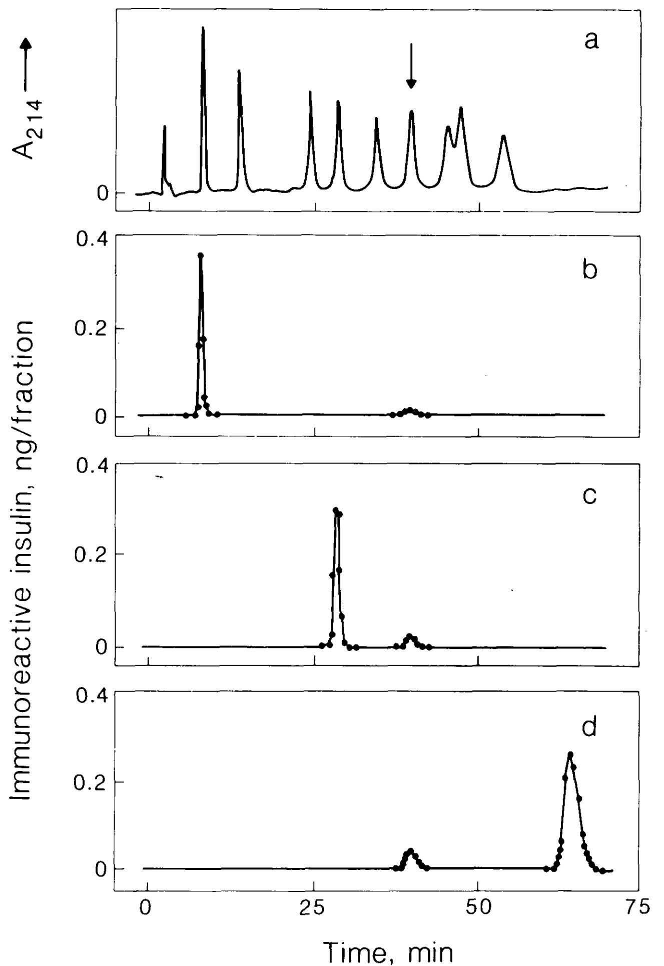

FIGURE 2. Reverse-phase HPLC separations of abnormal insulins isolated from the serum of human subjects. Panel a: a mixture of insulin standards visualized by optical absorbance at 214 nm; the peaks correspond, from left to right, to human [Ser^{B24}]insulin, human [Ser^{B25}]insulin, porcine [Ala^{B24}]insulin, human [Leu^{B25}]insulin, porcine [Leu^{B25}]insulin, normal human insulin, human [Leu^{B24}]insulin, normal porcine insulin, and porcine [Leu^{B24}]insulin, respectively. Panels b, c, and d: immunoaffinity-purified insulin isolated from the serum of three unrelated human subjects who secrete abnormal insulins. Insulin content was determined in all fractions by radioimmunoasay, but data points are shown only for those fractions where immunoreactive insulin content was above the limit of detection. Note that a small peak of normal insulin appears in each of panels b, c, and d. In all cases, separations occurred on a reverse-phase C-18 column using triethylammonium phosphate buffer containing $NaClO_4$.

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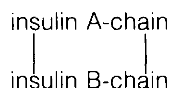
both termini (as for insulin[2,5,6]), or at a central or nonterminal location (as for glucagon[24,25]). A trypsin-like activity alone could serve for conversion of prosomatostatin to somatostatin (due to the COOH-terminal location of the hormone sequence in the precursor), whereas trypsin-like and carboxypeptidase B-like activities would be required for conversion of the other three precursors to their final products. Proteolytic events involving posttranslational cleavage are followed in the case of pancreatic polypeptide by COOH-terminal amidation of the product through a complex enzyme-catalyzed reaction.[21,26]

Although primary structure clearly plays an important role in directing the processing of peptide hormone precursors, an understanding of insulin biosynthesis also requires consideration of peptide folding and disulfide bond formation. The location of the insulin B-chain at the NH_2-terminus of proinsulin and of the insulin A-chain at the COOH-terminus of the precursor dictates that unimolecular folding and disulfide bond formation occur before excision of the connecting C-peptide by endo- and exoproteolytic events. Only by these sequential processes would the synthesis of a hormone with the general structure shown below be possible.



Although conversion of proinsulin requires many steps and involves many intermediate forms,[7–9] the process is extremely efficient with more than 90% of the precursor being converted to insulin before the delivery of secretion granule contents to the extracellular fluid. Nevertheless, each of these steps represents a potential site for genetic or metabolic error.

Abnormal human insulins. Any consideration of abnormal products of the human insulin gene must deal with (1) the possible biosynthesis of a mutant insulin bearing a single amino acid substitution, (2) the possibility of mutation altering the course of proinsulin conversion yielding a mutant intermediate of proinsulin processing, rather than a mutant insulin, (3) difficulty in obtaining sizable amounts of material, and (4) methods for detecting molecular alterations in secreted products of the insulin gene. We have used reverse-phase, high-pressure liquid chromatography (HPLC), a highly resolving separation technique, to identify insulins having single amino acid replacements. Panel a of Figure 2 illustrates the HPLC elution of nine natural and synthetic insulins from our isocratic reverse-phase system employing optical absorbance to detect each of these peptides in microgram amounts. It is notable that a variety of such analogues bearing amino acid substitutions at position B24, B25, or B30 are readily resolved from normal human insulin and from each other. Control studies showed that much lower, nanogram amounts of insulin were equally well separated and were readily recovered from the reverse-phase column when eluted material was detected by insulin radioimmunoassay.[15] These studies, as well as those involving the identification of normal insulin in the sera of normal subjects, permitted, in collaboration with Dr. Arthur H. Rubenstein, the application of HPLC separation and radioimmunometric analysis to the detection of abnormal insulins in man. Panels b, c, and d of Figure 2 show the elution profiles of immunoaffinity-purified serum insulin from individuals in three separate families. In each case, the individual showed hyperinsulinemia and hyperglycemia, and appeared to respond normally to the administration of exogenous insulin. Notably, the major fraction of immunoreactive insulin from each of these patients eluted at a unique position that was different from that of normal human insulin. Each could thus be identified as secreting an abnormal human insulin; serum from each of these three patients also appeared to contain lower amounts of a second insulin that was indistinguishable from normal human insulin.[12,13,15] These results are thus consistent with these patients expressing both normal and abnormal insulin gene alleles.

Our approach to identifying the structures of abnormal human insulins has relied on making comparisons between the natural, serum-derived hormones and synthetic insulin analogues with defined amino acid substitutions. Limited chemical analysis of pancreatic insulin from the subject identified in Figure 2c (derived from a 2-g pancreatic biopsy) suggested that the patient's abnormal insulin had arisen from a leucine for phenylalanine substitution at position B24 or B25.[13] Confirmation of the region of mutation by restriction endonuclease mapping of the patient's leukocyte-derived insulin genes,[27] prompted us to synthesize the relevant insulin analogues. In collaboration with Dr. Emil T. Kaiser, the analogues of human insulin, human [Leu^{B24}]insulin, and human [Leu^{B25}]insulin were prepared by trypsin-catalyzed peptide bond formation between the α-carboxyl group of Arg^{22} of bis-*t*-butyloxycarbonyl desoctapeptide insulin (derived from porcine insulin) and the α-amino groups of chemically synthesized COOH-terminal B-chain octapeptides containing leucine for phenylalanine substitutions at positions corresponding to B24 or B25;[28,29] this method, which was pioneered by Inouye et al.,[28] permits a synthesis of insulin analogues that would have been much more difficult had chemical methods alone been used. Subsequent detailed analysis showed that (1) human [Leu^{B24}]insulin and human [Leu^{B25}]insulin were readily separated by reverse-phase HPLC, (2) the patient's abnormal insulin (Figure 2c) migrated to the position of human [Leu^{B25}]insulin on the reverse-phase column, and (3) the patient's abnormal insulin and the [Leu^{B25}]insulin analogue co-chromatographed when the two were studied in a mixture;[15] the patient's abnormal human insulin was thus identified as human [Leu^{B25}]insulin and was given the designation human insulin B25 (Phe → Leu).

Identification of the amino acid substitution present in the abnormal insulin from the patient noted in Figure 2b presented a much more difficult problem: although the abnormal insulin was clearly more hydrophilic than normal insulin (as determined by its very early elution from the reverse-phase HPLC column) no data were available on potential chemical differences between this abnormal hormone and its normal counterpart. Restriction endonuclease mapping of the insulin genes from this patient, like those from the patient just described, however, showed the loss of an MboII restriction site normally occurring in the gene sequence corresponding to phenylalanine residues B24 and B25.[15] Localization of a possible mutation to this region, together with the presumption that a single nucleotide change had resulted in replacement of either Phe^{B24} or Phe^{B25} by a significantly more hy-

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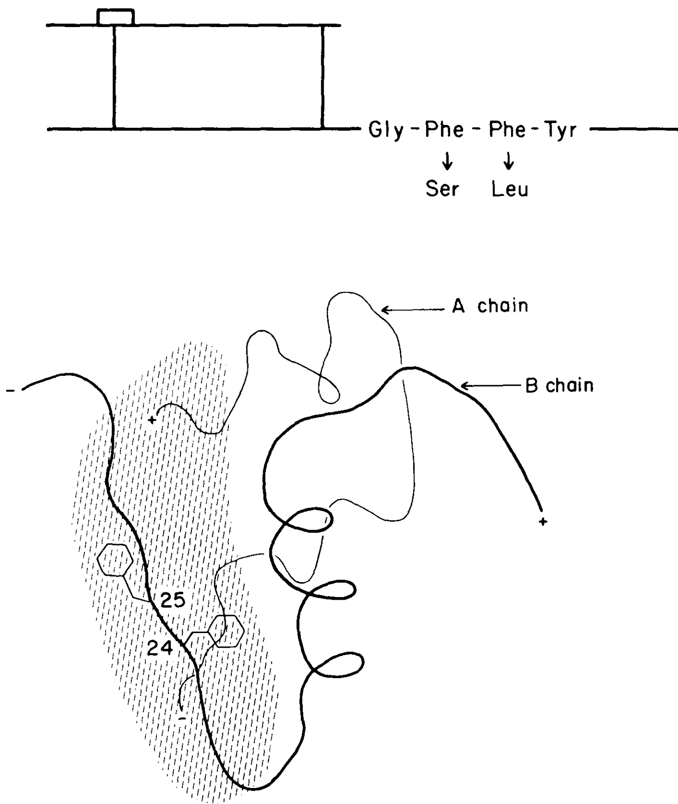

FIGURE 3. Diagrammatic structures of abnormal and normal human insulins. The upper portion of the figure illustrates the A- and B-chain portions of insulin and shows residues B23–B26 explicitly; these residues comprise an invariant tetrapeptide sequence in the COOH-terminal region of the insulin B-chain. Vertical arrows identify amino acid substitutions that occur separately in each of two abnormal human insulins (Phe B24 → Ser B24 and Phe B25 → Leu B25). The lower portion of the figure illustrates the structure of insulin as determined by x-ray crystallography. The side chains of phenylalanine residues B24 and B25 are shown explicitly. The shaded area illustrates the portion of insulin that is thought to be most important in conferring biologic activity to the hormone. The NH_2-termini of the insulin A- and B-chains are indicated by + whereas the COOH-termini are indicated by −.

drophilic residue, led to the prediction that one or the other of these aromatic residues had been replaced by serine. Using our earlier approach, both human [Ser^{B24}]insulin and human [Ser^{B25}]insulin were prepared by enzyme-catalyzed semisynthesis and their elution from reverse-phase HPLC columns was compared with that of the patient's abnormal insulin. The patient's insulin migrated to the position of, and co-chromatographed with, human [Ser^{B24}]insulin during HPLC analysis. On this basis, the patient's insulin (Figure 2a) was identified as human [Ser^{B24}]insulin and was designated human insulin B24 (Phe → Ser).[17] Importantly, the loci of genetic mutation in the patients found to secrete human [Leu^{B25}]insulin and human [Ser^{B24}]insulin have been identified by Dr. Donald F. Steiner and his colleagues using the techniques of molecular cloning and DNA sequence analysis.[30,31] Additional studies on members of the family of the subject identified in Figure 2a have documented expression of the gene coding for human [Ser^{B24}]insulin in the patient's father, two brothers, a sister, and a niece;[12] importantly, her mother appears to secrete only normal insulin. Although only two of these individuals are diabetic, it appears that all affected subjects codominantly express both normal and abnormal insulin gene alleles and that differential rates of clearance, rather than differential rates of biosynthesis or secretion,[12] account for the disparate steady-state concentrations of abnormal and normal hormones identified in the peripheral circulation of members of this family and other families where abnormal insulin gene products are secreted (Figure 2).

The line drawing of insulin at the top of Figure 3 illustrates the sites of amino acid substitution occurring in two of the three subjects whose insulin was examined in the HPLC studies of Figure 2. (The site of substitution in the third patient has yet to be identified, but it appears not to have occurred at position B24 or B25.[15]) In both patients b and c (in whom abnormal insulins were identified as human [Ser^{B24}]insulin and human [Leu^{B25}]insulin, respectively), amino acid substitution has occurred in the tetrapeptide sequence Gly^{B23}-Phe^{B24}-Phe^{B25}-Tyr^{B26}, a sequence that has remained invariant during animal evolution. Since rather drastic changes in the biologic activity of abnormal insulins must accompany expression of mutant insulin genes for such mutations to be phenotypically recognizable, it is not surprising that amino acid substitutions in insulins from these patients (as well as perhaps in the insulin from the third patient) would occur at invariant residues; amino acid side chains at these positions must play especially important roles in determining the receptor binding characteristics and biologic effectiveness of the hormone. In fact, [Leu^{B24}]insulin and [Ser^{B24}]insulin retain only about 15% of the potency of normal insulin, whereas [Leu^{B25}]insulin and [Ser^{B25}]insulin retain only about 2% of the potency of the normal hormone[17,18,29,32] in vitro; [Leu^{B24}]insulin has also been shown to have decreased biologic activity in vivo.[33]

Although the synthesis of position B24- and position B25-substituted insulins was originally undertaken in order to

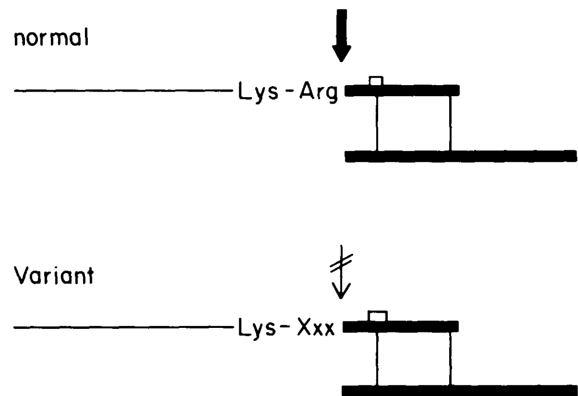

FIGURE 4. Diagrammatic structures of normal and abnormal type II intermediates of proinsulin conversion. The upper portion of the figure illustrates the structure of the normal intermediate; the usual Lys-Arg sequence at the prohormone conversion site is shown explicitly. The lower portion illustrates the proposed structure of the abnormal intermediate found in individuals with familial hyperproinsulinemia; the lysine residue present at the conversion site remains, but the arginine residue has been replaced by an unidentified, nonbasic amino acid. Both structures begin with the C-peptide at the far left connected to the insulin A-chain; the A-chain is disulfide-bonded to the unextended insulin B-chain.

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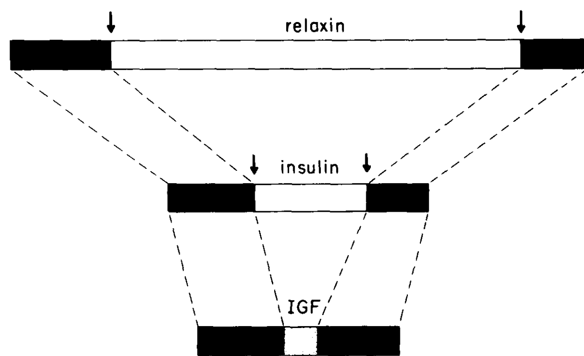

FIGURE 5. Diagrammatic structures of precursors for insulin-related peptides. Homologous regions of relaxin, insulin, and insulin-like growth factor are shown as solid bars. Amino acid sequences connecting B-chain and A-chain sequences in the precursors for relaxin and insulin are shown as open bars; these sequences are removed during processing of the precursors to their corresponding two-chained products (vertical arrows). The amino acid sequence of insulin-like growth factor that corresponds to these connecting peptides, but which is not removed by proteolytic processing events, is shown as a stippled bar; insulin-like growth factor is a single-chained peptide hormone.

identify abnormal products of the human insulin gene, use of these hormone analogues has provided important information on insulin structure-function relationships. Thus, the relative biologic potencies of the analogues noted above demonstrate (1) that substitution at position B25 has a significantly more marked biologic effect than substitution at position B24 and (2) that equivalent activities are obtained when the aromatic side chain of phenylalanine is replaced by the branched, hydrophobic side chain of leucine or by the small, hydrophilic side chain of serine. Additional studies have shown that B25-substituted analogues appear to retain a normal conformation, whereas those substituted at position B24 have altered conformations that, for example, decrease their abilities to dimerize.[34,35] The lower part of Figure 3 illustrates the different contributions of the side chains of Phe^{B24} and Phe^{B25} to the structure of insulin. The side chain at position B25 occurs on the exterior of the molecule and likely has direct contact with the cell-surface receptor on insulin responsive tissues;[17,18,34,35] as replacement of this residue by either leucine or serine causes equivalently decreased biologic activity, it is probable that aromaticity at this position per se is an important prerequisite for insulin-receptor interactions and that π orbital bonding may play a role in determining the affinity of the receptor for the hormone. On the other hand, the side chain at position B24 points more inwardly and would appear to have less opportunity for direct receptor contact. It is probable that substitutions at this position alter the orientation of the COOH-terminal part of the insulin B-chain relative to the rest of the insulin molecule and that potential interactions of the hormone with receptor are altered in an unfavorable way.

Abnormal human proinsulin intermediates. As noted earlier, mutation-induced amino acid substitutions at sites other than those associated with the 21-residue insulin A-chain and the 30-residue insulin B-chain could have important consequences in terms of the eventual biosynthesis of active insulin. For example, substitutions in proinsulin at the paired dibasic amino acid residues joining the insulin B-chain to the C-peptide (Arg-Arg) or at those joining the C-peptide to the insulin A-chain (Lys-Arg) could hinder conversion of the precursor to its normal product. The syndrome called familial hyperproinsulinemia[36] documents this possibility. Subjects with this autosomal dominant characteristic appear to be hyperinsulinemic, but the major fraction of serum insulin-like immunoreactivity in such individuals has a molecular weight similar to that of proinsulin rather than that of insulin. These individuals, like those expressing abnormal insulins, show no signs of insulin resistance; they may or may not exhibit mild hyperglycemia.[14,16,36]

Although it has been clear for some time that the insulin-like material in the serum of subjects with familial hyperproinsulinemia is a two-chained intermediate of proinsulin conversion (rather than proinsulin itself), structural studies have been hindered by the low amounts of material available and by the multiplicity of possible intermediate forms.[9] Nevertheless, the multiple techniques of gel filtration, polyacrylamide gel electrophoresis, oxidative sulfitolysis, HPLC, and radioimmunoassay (for insulin, C-peptide and *S*-sulfo-insulin B-chain) have now shown that the proinsulin-like material in two kindreds with familial hyperproinsulinemia is a two-chained proinsulin intermediate in which the C-peptide remains attached to the insulin A-chain.[14,16] The upper portion of Figure 4 illustrates the structure of a normal type II proinsulin intermediate having these structural characteristics. This intermediate (which would arise from cleavage of proinsulin at the dibasic amino acid pair between the B-chain and the C-peptide), as well as the type I intermediate (which would arise from cleavage of proinsulin at the dibasic amino

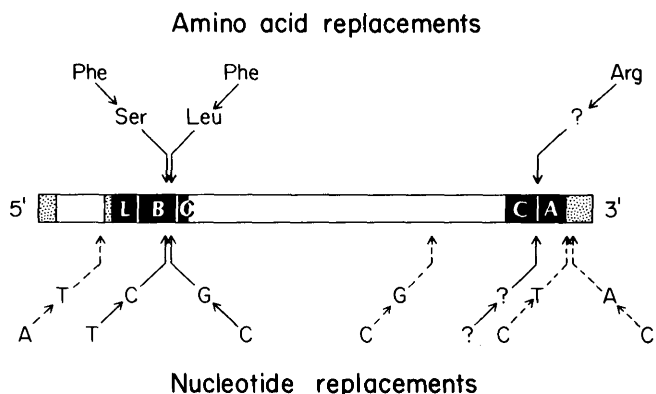

FIGURE 6. Diagrammatic structure of the human insulin gene. Shaded areas correspond to untranslated regions of the corresponding mRNA, open regions correspond to intervening sequences, and filled regions correspond to coding sequences. L, B, C, and A identify coding sequences for the leader (or signal) peptide, the insulin B-chain, the C-peptide, and the insulin A-chain, respectively. Note that the coding sequence for the C-peptide is split by an intervening sequence. Amino acid replacements identified as resulting from mutations within the insulin gene in subjects secreting abnormal gene products are shown above the gene structure; nucleotide changes that correspond to these amino acid replacements are shown below the gene structure (solid arrows). Dashed arrows indicate nucleotide changes that have been identified in the insulin genes of normal individuals; these replacements occur in intervening sequences and noncoding regions exclusively. The diagrammatic structure is drawn to scale.

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acid pair between the C-peptide and A-chain), is known to be produced during the normal processing of proinsulin to insulin.[2,3,8]

As the proinsulin intermediate in both kindreds with familial hyperproinsulinemia was apparently not converted to insulin by endogenous processing enzymes, it seemed probable that the abnormal intermediates resulted from an amino acid substitution at the dibasic amino acid conversion site normally joining the C-peptide to the insulin A-chain. Using the picomole amounts of the intermediate that could be isolated from serum by immunoaffinity chromatography, we were able to probe the structure at this site through chemical modification and enzymatic digestion: as the site was subject to cleavage by trypsin in the native peptide, but was not subject to tryptic cleavage after peptide α- and ϵ-amino groups had been acetylated with acetic anhydride, we deduced that Lys^{64} was present in the usual conversion site, but Arg^{65} had been replaced by a nonbasic amino acid residue.[14,16] The proposed structure of the abnormal proinsulin intermediate that results from mutation in the human insulin gene in both kindreds with familial hyperproinsulinemia is shown in the lower portion of Figure 4. Although the identity of the amino acid replacing Arg^{65} has yet to be determined, our findings imply that sequences of paired dibasic amino acids are indeed necessary for a peptide hormone precursor to be recognized by endogenous converting enzymes and for normal processing to occur. This finding thus augments related findings based on abnormal products of the human albumin gene: abnormal human proalbumins Christchurch and Lille contain the sequences Arg-Glu and His-Arg, respectively, at the proprotein conversion site, rather than the normal sequence Arg-Arg.[37,38] These abnormal protein precursors (which arise in liver), like the abnormal proinsulin intermediates (which arise in the pancreatic B-cell), are not converted to their normal products by the appropriate biosynthetic processing enzymes.

Although insulin gene mutations resulting in noncleavable intermediates of proinsulin conversion have their greatest impact in explaining the syndrome of familial hyperproinsulinemia, they also provide clues on the importance of amino acid substitution in the evolution of peptide hormone precursors and peptide hormones. Figure 5 illustrates diagrammatic structures for the precursors of three clearly related hormones: relaxin, insulin, and insulin-like growth factor.[39] In all three cases, these peptides contain homologous B-chain and A-chain regions (at their amino and carboxyl termini, respectively) connected by intervening segments. Only in the first two cases, however, is the intervening segment bounded by sites for precursor processing (whether these sites contain paired dibasic amino acids or other signals) and only in these examples is the segment removed by limited proteolysis to yield a two-chained hormone product. The potential "precursor" for insulin-like growth factor does not contain appropriate conversion sites,[40] and insulin-like growth factor remains as a single peptide chain. Considerations of structural homology suggest that discontiguous gene duplications followed by mutation could have resulted in ancestral genes for these three important peptide effectors. Although all mutation-derived amino acid substitutions would have potential structural and functional consequences, amino acid substitutions leading toward or away from precursor conversion sites would have special importance: a very limited number of mutations resulting in such amino acid substitutions would determine the size and overall structure of the product and whether or not the hormone contained one or two peptide chains. Evolution has clearly selected for small, two-chained peptide hormones in the cases of relaxin and insulin and for a single-chained peptide hormone in the case of insulin-like growth factor. Although abnormal human proinsulins lacking the correct dibasic amino acid conversion site clearly represent peptides with deteriorated function, one can imagine that similar large forms might have represented the products of ancestral genes that were evolving in the direction of directing the synthesis of insulin as we know it.

CONCLUSIONS

It is clear from these studies that the insulin gene, like the genes for a variety of other peptides and proteins, is subject to mutations that result in abnormal peptide products having altered function. Figure 6 illustrates the schematic structure of the human insulin gene[5,6] and documents the complexity of its components including (1) the 5′ untranslated region (split by an intervening sequence), (2) the segment coding for the leader or signal peptide, (3) the segment coding for the B-chain, (4) the segment coding for the C-peptide (also split by an intervening sequence), (5) the segment coding for the A-chain, and finally (6) the 3′ untranslated region. As illustrated in the figure, a variety of mutations resulting in both abnormal insulins and abnormal intermediates of proinsulin conversion have been documented, as have "silent" single nucleotide changes that occur in the intervening sequences and noncoding regions of genes from normal individuals. Although we are not yet sure of the incidence of insulin gene mutations in man, it is probable that mutations in 5′ untranslated regions (affecting the insulin gene promotor or the eventual interaction of the corresponding mRNA with ribosomes), in the sequences corresponding to splice junctions (for removal of intervening sequences and mRNA maturation), in the coding segment for the leader sequence (affecting the intracellular translocation of the newly synthesized gene product), and in any nonintervening segment where a sense codon has changed to a stop codon (leading to premature peptide termination) also occur, and that they would have detrimental effects in the overall biosynthesis of insulin. Thus, while some mutations could be phenotypically neutral (for example, those at evolutionarily variable sites), many will result in abnormal gene products and will contribute to physiologic abnormalities. Study of the abnormal products of the human insulin gene will continue to provide unique opportunities for examining the importance of structure in insulin biosynthesis, action, and metabolic clearance, as well as for probing the physiologic consequences of abnormal insulin gene expression in man.

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