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Possible Involvement of the A^{20}–A^{21} Peptide Bond in the Expression of the Biological Activity of Insulin. 3. [21-Desasparagine,20-cysteine ethylamide-A]insulin and [21-Desasparagine,20-cysteine 2,2,2-trifluoroethylamide-A]insulin†

Ying-Chi Chu, Run-Ying Wang, G. Thompson Burke, Jacob D. Chanley, and Panayotis G. Katsoyannis*
Department of Biochemistry, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029
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ABSTRACT: We have synthesized [21-desasparagine,20-cysteine ethylamide-A]insulin and [21-desasparagine,20-cysteine 2,2,2-trifluoroethylamide-A]insulin, which differ from natural insulin in that the C-terminal amino residue of the A chain, asparagine, has been removed and the resulting free carboxyl group of the A^{20} cysteine residue has been converted to an ethylamide and a trifluoroethylamide group, respectively. [21-Desasparagine,20-cysteine ethylamide-A]insulin displayed equivalent potency in receptor binding and biological activity, ca. 12% and ca. 14%, respectively, relative to bovine insulin. In contrast, [21-desasparagine,20-cysteine 2,2,2-trifluoroethylamide-A]insulin displayed a divergence in these properties, ca. 13% in receptor binding and ca. 6% in biological activity. This disparity is ascribed to a difference in the electronic state of the A^{20}–A^{21} amide bond in these two analogues. A model is proposed to account for the observation of divergence between receptor binding and biological activity in a number of synthetic insulin analogues and naturally occurring insulins. In this model, changes in the electronic state and/or the orientation of the A^{20}–A^{21} amide bond can modulate biological activity independently of receptor binding affinity. The A^{20}–A^{21} amide bond is thus considered as an important element in the "message region" of insulin.

In the previous two papers (Chu et al., 1987a,b), we have implicated A^{20}–A^{21} amide bond as a necessary structural feature for the expression of the biological activity of insulin and have suggested that this activity is modulated by the electronic state of the A^{20}–A^{21} amide bond. This may represent an important element of the "message region" of insulin. To further probe this speculation, we here describe the synthesis and biological evaluation of [21-desasparagine,20-cysteine ethylamide-A]insulin ([des-A^{21}Asn,A^{20}cysteine ethylamide]insulin) and [21-desasparagine,20-cysteine 2,2,2-trifluoroethylamide-A]insulin, ([des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin),[1] in which the C-terminal residue, asparagine, of the A chain has been eliminated and the resulting free carboxyl group at position A^{20} has been converted to an ethylamide and a trifluoroethylamide, respectively.

Experimental Procedures and Results

Details of materials, analytical procedures, and biological evaluation by lipogenesis assays in isolated rat fat cells, receptor binding assays in rat liver plasma membranes, and radioimmunoassays are given in a previous publication (Chu et al., 1987a). Receptor binding potency in isolated rat fat cells was examined as previously described (Burke et al., 1980).

General Aspects of Synthesis of [Des-A^{21}Asn,A^{20}cysteine ethylamide]- and [Des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulins. The synthesis of these analogues was carried out by the interaction of the S-sulfonated bovine (sheep) B chain with the respective S-sulfonated A chain analogue as described previously (Chu et al., 1987a). The overall procedure for the preparation of the S-sulfonated A chain analogues is comparable to that outlined previously (Chu et al., 1987a).

*Glycyl-*L*-isoleucyl-*L*-valyl-*L*-glutamyl-*L*-glutaminyl-S-sulfo-*L*-cysteinyl-S-sulfo-*L*-cysteinyl-*L*-alanylglycyl-*L*-valyl-S-sulfo-*L*-cysteinyl-*L*-seryl-*L*-leucyl-*L*-tyrosyl-*L*-glutaminyl-*L*-leucyl-*L*-glutamyl-*L*-asparaginyl-*L*-tyrosyl-S-sulfo-*L*-cysteine*

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[1] Abbreviations: AcOH, acetic acid; CM, carboxymethyl; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoramide; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; TEA, triethylamine; THF, tetrahydrofuran; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride. Compounds designated by Roman numerals are described fully in the supplementary material (see paragraph at end of paper regarding supplementary material). The IUPAC–IUB name of [21-desasparagine,20-cysteine ethylamide-A]insulin, for example, is des-A21-asparagine-[20-cysteine ethylamide]insulin (des-Asn^{A21}-[A20-cysteine ethylamide]insulin).

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Table I: Amino Acid Composition[a] of an Acid Hydrolysate and an Enzymatic Digest (Leucine Aminopeptidase) of the S-Sulfonated [Des-A^{21}Asn,A^{20}cysteine ethylamide]A and [Des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]A Chains

	ethylamide analogue				trifluoroethylamide analogue			
	acid hydrolysis		enzymatic hydrolysis		acid hydrolysis		enzymatic hydrolysis	
amino acid	theory	found	theory	found	theory	found	theory	found
Asp	1	1	0	0	1	1	0	0
Ser	1	1	1	*b*	1	1	1	*b*
Asn	0	0	1	*b*	0	0	1	*b*
Gln	0	0	2	*b*	0	0	2	*b*
Glu	4	4.1	2	1.9	4	4	2	1.9
Gly	2	1.8	2	1.9	2	1.9	2	2
Ala	1	0.9	1	0.8	1	1	1	1
$^1/_2$-Cys	4	nd[c]	0	0	4	nd[c]	0	0
Val	2	1.5	2	1.7	2	1.6	2	1.9
Ile	1	0.4	1	0.8	1	0.6	1	0.7
Leu	2	2	2	2	2	2.2	2	2.1
Tyr	2	2	2	2	2	2	2	2
S-sulfo-Cys	0	0	4	3.2[d]	0	0	4	2.9[d]

[a] Number of amino acid residues per molecule. [b] Emerge on the same position and not determined. [c] nd, not determined. [d] The substituted cysteinamide is not hydrolyzed by the enzyme.

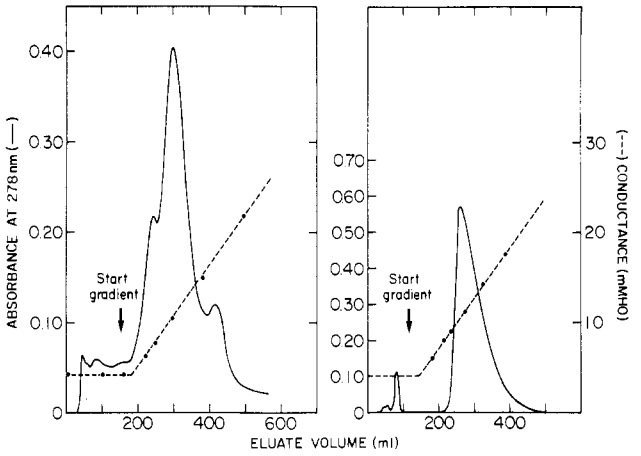

FIGURE 1: (Left) Chromatography of crude [des-A^{21}Asn,A^{20}cysteine ethylamide]A chain *S*-sulfonate on a 1.2 × 40 cm Cellex-E column with 0.1 M Tris-HCl buffer (pH 7.0) and a linear NaCl gradient. The effluent was monitored with an ISCO spectrophotometer and a conductivity meter (Radiometer, Copenhagen). (Right) Rechromatography of the product obtained after dialysis and lyophilization of the main peak effluent (270–377 mL) depicted in the left panel.

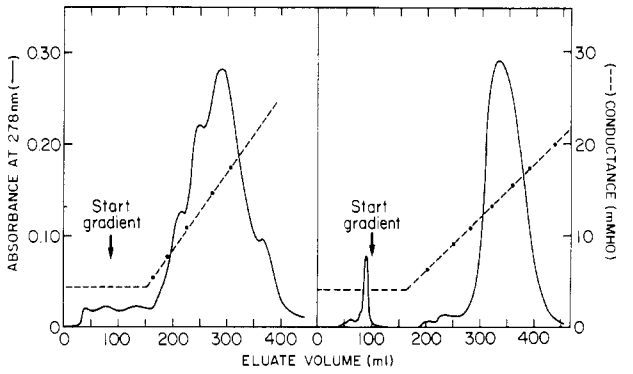

FIGURE 2: (Left) Chromatography of crude [des-A^{21}Asn,A^{20}cyteine trifluoroethylamide]A chain *S*-sulfonate. (Right) Rechromatography of the product obtained after dialysis and lyophilization of the main peak effluent (264–372 mL) depicted in the left panel. Chromatography conditions as in Figure 1.

Ethylamide (Sheep Insulin [Des-A^{21}Asn,A^{20}cysteine ethylamide]A Chain S-Sulfonate) (IX). The deblocking of the protected eicosapeptide VIII (238 mg) upon treatment with 1 M TFMSA in TFA (9 mL) containing thioanisole (1.8 mL) and *m*-cresole (1.3 mL) and the conversion of the resulting reduced product to the S-sulfonated derivative were carried out as described previously (Chu et al., 1987a). After Sephadex G-15 chromatography, 178 mg of crude S-sulfonated A chain analogue was obtained. For purification, this product (92 mg) was chromatographed on a Cellex-E column (1.2 × 40 cm) with 0.1 M Tris-HCl buffer (pH 7.0) and a linear NaCl gradient as described previously (Chu et al., 1987a). The eluate under the major peak (270–377 mL) was dialyzed and lyophilized to give 55 mg of product (Figure 1). This material (107 mg) was rechromatographed on the same column and under identical conditions, and from the eluate under the main peak (231–381 mL) (Figure 1) after dialysis and lyophilization the purified [des-A^{21}Asn,A^{20}cysteine ethylamide]A chain *S*-sulfonate was obtained, weight 92.4 mg.

On thin-layer electrophoresis (Chu et al., 1987a) in 2 N acetic acid–0.6 N formic acid (1:1 v/v), pH 2.0 and 480 V, the synthetic chain moved as a single band (data not shown). Amino acid analysis of the A chain analogue after either acid hydrolysis or enzymatic digestion with leucine aminopeptidase gave a composition, expressed in molar ratios, in good agreement with the theoretically expected values (Table I).

Glycyl-L-isoleucyl-L-valyl-L-glutamyl-L-glutaminyl-S-sulfo-L-cysteinyl-S-sulfo-L-cysteinyl-L-alanylglycyl-L-valyl-S-sulfo-L-cysteinyl-L-seryl-L-leucyl-L-tyrosyl-L-glutaminyl-L-leucyl-L-glutamyl-L-asparaginyl-L-tyrosyl-S-sulfo-L-cysteine Trifluoroethylamide (Sheep Insulin [Des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]A Chain S-Sulfonate) (IXa). From 236 mg of the protected eicosapeptide VIIIa after deblocking, sulfitolysis, and Sephadex G-15 chromatography, 150 mg of crude [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]A chain *S*-sulfonate was obtained. For purification, this material (73 mg) was chromatographed on a Cellex-E column under the conditions described above, and from the effluent under the major peak (264–370 mL) (Figure 2) after dialysis and lyophilization 33 mg of product was obtained. Rechromatography of this material (49 mg) on the same column and under the same conditions as above (Figure 2) afforded the purified [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]A chain *S*-sulfonate (285–400 mL of effluent, 40 mg).

On thin-layer electrophoresis, as above, the synthetic chain moved as a single component (data not shown). Amino acid analysis after either acid hydrolysis or enzyme digestion gave a composition expressed in molar ratios in good agreement with the theoretically expected values (Table I).

Synthesis and Isolation of Sheep [Des-A^{21}Asn,A^{20}cysteine ethylamide]insulin. The synthesis of this analogue by the combination of the S-sulfonated derivatives of sheep B chain

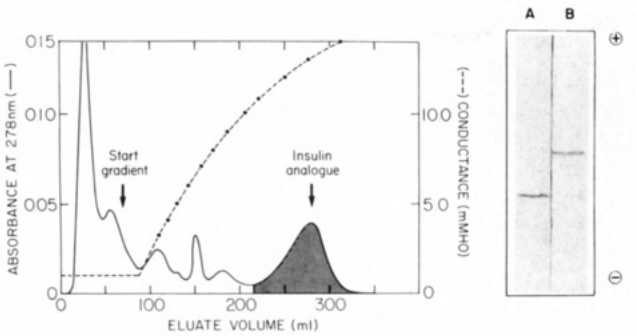

FIGURE 3: (Left) Chromatography of a combination mixture of bovine (sheep) B chain *S*-sulfonate and [des-A^{21}Asn,A^{20}cysteine ethylamide]A chain *S*-sulfonate on a 0.9 × 23 cm CM-cellulose column with acetate buffer (Na^+, 0.024 M, pH 3.3) and an exponential NaCl gradient. The column effluent was monitored with an ISCO spectrophotometer and a conductivity meter. The insulin analogue (221–321 mL of effluent) was recovered as the hydrochloride. (Right) Paper print of thin-layer isoelectric focusing of synthetic [des-A^{21}Asn,A^{20}cysteine ethylamide]insulin (A) and natural bovine insulin (B) in a 1:1 mixture of pH 3–10 and pH 4–6 ampholytes on a 20-cm separation distance. Focusing: constant power; 8 W for 4 h.

Table II: Amino Acid Composition[a] of an Acid Hydrolysate of Sheep [Des-A^{21}Asn,A^{20}cysteine ethylamide]- and [Des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin

	ethylamide analogue		trifluoroethyl-amide analogue	
amino acid	theory	found	theory	found
Lys	1	1.1	1	1
His	2	1.8	2	2.1
Arg	1	1.3	1	1.3
Asp	2	2.2	2	2
Thr	1	0.9	1	0.7
Ser	2	1.8	2	1.6
Pro	1	1.1	1	1.3
Glu	7	7.2	7	7
Gly	5	4.7	5	4.9
Ala	3	2.7	3	2.8
$^1/_2$-Cys	6	nd[b]	6	nd[b]
Val	5	4.7	5	4.8
Ile	1	0.5	1	0.7
Leu	6	6	6	6.3
Tyr	4	3.5	4	3.8
Phe	3	2.8	3	2.7

[a] Number of amino acid residues per molecule. [b] nd, not determined.

and [des-A^{21}Asn,A^{20}cysteine ethylamide]A chain and its purification were accomplished by exactly the same procedure as described in the previous paper (Chu et al., 1987a). Chromatography of a combination mixture of 10.7 mg of S-sulfonated sheep B chain and 20.4 mg of [des-A^{21}Asn,A^{20}cysteine ethylamide]A chain *S*-sulfonate (4.5 mL of 0.1 M glycine buffer, pH 10.5, 3.7 mg of dithiothreitol) gave the pattern shown in Figure 3. The insulin analogue was isolated from the effluent (221–321 mL) via picrate (Katsoyannis et al., 1967) as the hydrochloride (1.8 mg).

Amino acid analysis of an acid hydrolysate of this analogue gave the molar ratios shown in Table II in agreement with the theoretically expected values. On isoelectric focusing on thin-layer plates in a 1:1 mixture of pH 3–10 and pH 4–6 ampholytes the synthetic analogue focused in one band (Figure 3).

Synthesis and Isolation of Sheep [Des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin. For the synthesis of this analogue, 10 mg of sheep B chain *S*-sulfonate and 20.1 mg of [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]A chain *S*-sulfonate were combined (3.7 mg of dithiothreitol, 4.5 mL of 0.1 M glycine buffer, pH 10.5) as usual (Chu et al., 1987a). Chromatography of the reaction mixture gave the pattern

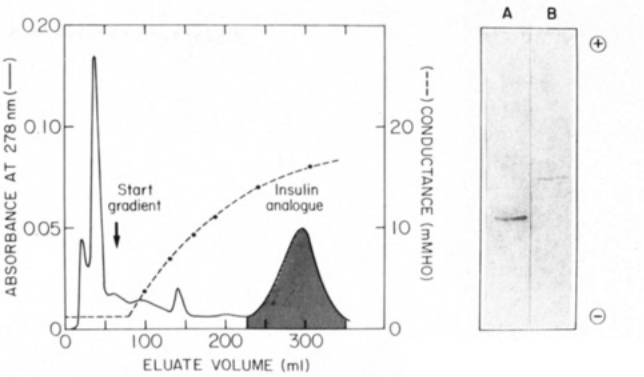

FIGURE 4: (Left) Chromatography of a combination mixture of the S-sulfonated bovine (sheep) B chain with the S-sulfonated [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]A chain. The figure depicts the chromatographic pattern obtained from the chromatography of ca. half of the combination mixture (see Experimental Procedures and Results). (Right) Paper print of thin-layer isoelectric focusing of synthetic [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin (A) and natural bovine insulin (B). Chromatography and isoelectric focusing conditions are the same as in Figure 3.

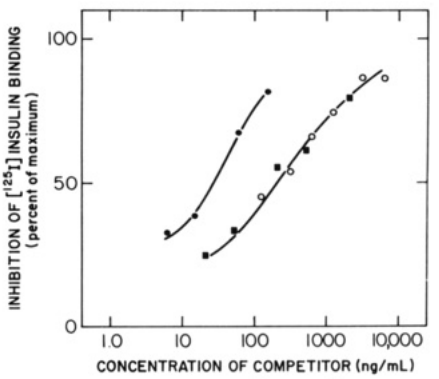

FIGURE 5: Effect of insulin (●), [des-A^{21}Asn,A^{20}cysteine ethylamide] insulin (○), and [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin (■) on the binding of insulin to rat liver plasma membranes. Inhibition of binding, expressed as percent of maximum, is presented as a function of the concentration of the competitor. The data represent the means of triplicate determinations in representative separate experiments that were performed 3 times for each analogue. ^{125}I-Insulin bound in the absence of competitor amounted to 4% of the input radioactivity.

shown in Figure 4. The insulin analogue was isolated from the effluent as the hydrochloride (5.1 mg).

Amino acid analysis of an acid hydrolysate of the purified analogue gave the molar ratios shown in Table II in agreement with the theoretically expected values. On isoelectric focusing, as above, the synthetic analogue focused in one band (Figure 4).

Biological Evaluation of the Synthetic Insulin Analogues. Figure 5 shows the effect of varying concentrations of bovine insulin, [des-A^{21}Asn,A^{20}cysteine ethylamide]insulin, and [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin upon the binding of ^{125}I-insulin to rat liver plasma membranes. The potencies of the two analogues, relative to bovine insulin, were essentially identical, 12.3 ± 3% and 13.1 ± 3.3%, respectively.

Figure 6 shows that, in contrast, the relative potencies for the analogues in lipogenesis assays were quite different from each other. [Des-A^{21}Asn,A^{20}cysteine ethylamide]insulin showed a potency of 13.9 ± 0.5% relative to bovine insulin, a figure in close agreement with its receptor binding affinity. [Des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin, however, showed a relative potency of 6.2 ± 0.9%, more than 50% lower than its receptor binding affinity. The difference in potency obtained from receptor binding assays and lipogenesis assays for the latter analogue was statistically highly significant (*p*

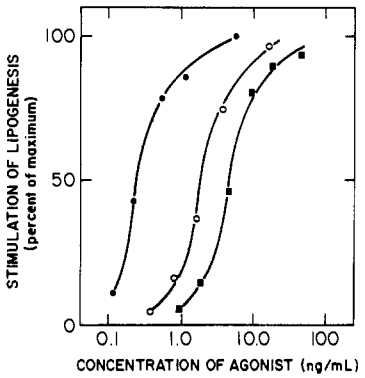

FIGURE 6: Effect of insulin (●), [des-A^{21}Asn,A^{20}cysteine ethylamide]insulin (○), and [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin (■) on the stimulation of lipogenesis in isolated rat adipocytes. Stimulation, expressed as percent of maximum, is presented as a function of the concentration of agonist. The data represent the means of triplicate determinations in representative separate experiments, which were performed 3 times for each analogue. Zero and 100% stimulation amounted respectively to 5.4 and 78.8 nmol of [3-^{3}H]glucose (mg of cells)$^{-1}$ h^{-1}.

< 0.02 by Student's t test). The substitution of the trifluoroethyl moiety for the ethyl moiety resulted in a divergence between receptor binding affinity and biological activity.

To control for a possible difference between behavior in a liver-derived assay versus an adipocyte assay, receptor binding assays in isolated fat cells were performed with [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin (data not shown). The calculated potency for the analogue was 11.4% relative to bovine insulin, not significantly different from the figure obtained in liver membranes.

In radioimmunoassay (data not shown), the analogues differed in potency from each other, by almost 50%; the trifluoroethylamide analogue assayed at 2.5% while the unsubstituted ethylamide analogue assayed at 4.7%, both relative to bovine insulin.

Discussion

In a previous paper (Chu et al., 1987a) we presented data indicating that amidation of the A^{20} carboxyl group of [des-A^{21}Asn]insulin results in analogues displaying significant biological activity. We concluded from this finding in conjunction with the relative inactivity of [des-A^{21}Asn]insulin (Chu et al., 1987a) that the A^{20}–A^{21} amide moiety, properly oriented within the insulin molecule, plays an important role in the expression of the biological activity of this hormone. In the preceding paper (Chu et al., 1987b), we suggested that the electronic state of the A^{21} residue in the insulin molecule modulates the biological activity independently of the receptor binding affinity of this hormone. We speculated then that this modulation may be the result of an inductive field effect of the A^{21} residue on the electronic state (dipolar form) of the A^{20}–A^{21} amide bond. On the basis of these considerations, we have speculated (Chu et al., 1987a,b) that the A^{20}–A^{21} amide bond is a significant constituent of the "message region" of insulin; it is involved in the expression of the biological activity and in the modulation of biological activity independently of receptor binding affinity. The data presented in this paper are consistent with this speculation.

We have synthesized [des-A^{21}Asn,A^{20}cysteine ethylamide]- and [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin and evaluated their biological behavior. The A^{20} ethylamide analogue displayed in receptor binding and in lipogenesis assays potencies of ca. 12% and ca. 14%, respectively, relative to bovine insulin. The A^{20} trifluoroethylamide analogue exhibited potencies of ca. 13% and ca. 6% in receptor binding and lipogenesis assays, respectively, relative to the natural hormone. It is apparent that whereas the A^{20} ethylamide analogue exhibits equivalent receptor binding and biological potencies, the A^{20} trifluoroethylamide analogue displays a significant divergence between receptor binding and biological potencies. We have already reported (Burke et al., 1980) that [21-asparaginamide-A]insulin also exhibits a significant divergence between receptor binding (ca. 60%) and biological activity (ca. 13%), relative to bovine insulin. Since both the A^{20} ethylamide and A^{20} trifluoroethylamide analogues show the same binding affinity to the insulin receptor, the marked difference (ca. 50%) in biological activity may be attributed to a difference in the nature of the A^{20} amide bonds. The trifluoromethyl group is a relatively strong electron-attracting moiety, whereas the unsubstituted methyl group is an electron-repelling moiety. It can then be reasonably concluded that the dipolar character of the A^{20} amide bond ($—C(O^-)=N^+HR$; R = $-CH_2CH_3$ vs $-CH_2CF_3$) is significantly reduced in the trifluoroethylamide as compared to the A^{20} ethylamide analogue. We have suggested that a comparable situation exists between [21-asparaginamide-A]insulin, which exhibits disparity between receptor binding and biological activity, and [21-asparagine diethylamide-A]insulin, which displays equivalent receptor binding and biological activity (ca. 48% and ca. 56%, respectively, relative to bovine insulin) (Chu et al., 1987b). Apparently, then, the electronic state (dipolar form) of the A^{20}–A^{21} amide bond does indeed play a significant role in selectively modulating biological activity independently of receptor binding affinity.

From the data presented in the two preceding papers (Chu et al., 1987a,b) and this paper, a reasonable generalized rationale emerges regarding the role of the A^{20}–A^{21} amide bond in the expression of the biological activity of insulin and its analogues. In the large majority of insulin analogues, biological activity seems to be a direct function of receptor binding affinity. In these cases structural changes that alter the receptor binding affinity result in a comparable change in biological activity. Equivalence between binding affinity and biological activity requires that the intrinsic ability of a receptor-bound agonist molecule to generate a biological event be the same as that of a receptor-bound molecule of natural insulin. Divergence of these properties, on the other hand, requires that the intrinsic ability of a bound agonist molecule be different from that of insulin. We have reported instances in which this intrinsic activity appears higher as in [21-proline-B]insulin (Schwartz et al., 1983) and lower as in [21-asparaginamide-A]insulin and [des-A^{21}Asn,A^{20}cysteine trifluoroethylamide]insulin than is the case of natural insulin. Lower intrinsic activity is also a property of porcupine (Horuk et al., 1980) and hagfish insulins (Emdin et al., 1980). We suggest that most modified insulins apparently exhibit the same orientation and dipolar character of the A^{20}–A^{21} amide bond upon binding to the receptor as does insulin, consonant with the observation that changes in binding affinity are accompanied by equivalent changes in biological activity. Divergence between these properties would be expected to occur if the orientation and/or dipolar state of the A^{20}–A^{21} amide bond were different from that of natural insulin. The divergence between binding affinity and biological activity of [21-proline-B]insulin and porcupine and hagfish insulins, which are identical with bovine insulin in the A^{19}–A^{21} region, might be primarily attributed to a change of orientation of the A^{20}–A^{21} bond, imposed by the constraints of the changes in the primary structures of these molecules. On the other hand, the divergence in [21-asparaginamide-A]- and [des-A^{21}Asn,A^{20}cysteine

trifluoroethylamide]insulins might be attributed to a change in the electronic state of this bond. We recognize that the reappearance of biological activity in [des-A^{21}Asn]insulins resulting from amidation of the A^{20} carboxyl group (Chu et al., 1987a) could result from the elimination of a negative charge in an unfavorable position and/or from the restoration of the A^{20}–A^{21} amide hydrogen, which is apparently involved in the maintenance of the structure of insulin by hydrogen bonding to the carbonyl oxygen of the B^{23} Gly residue (Blundell et al., 1972; Chu et al., 1987a), resulting in an increase in receptor binding. However, we have shown that the A^{20}–A^{21} amide bond is involved in the modulation of biological activity independently of receptor binding. It thus appears that this amide moiety is a significant element of the message region of insulin and it can be logically concluded that it is a direct participant in the expression of the biological activity as well. In this regard it is of interest to note that Cutfield et al. (1986) have recently presented evidence indicating that the C-terminal region of the A chain of insulin may well be involved in the expression of the biological activity of this hormone.

While an amide is generally not a reactive moiety, under propitious conditions its dipolar form could exhibit greater reactivity. For example, it could act as a nucleophile. Indeed, the nucleophilic character of the oxygen atom of the dipolar form of amides has been demonstrated (Capon, 1964; Cohen & Witkop, 1974). The dipolar form could form a strong hydrogen bond with the receptor. One could then hypothesize that the dipolar form of the A^{20}–A^{21} amide bond, optimally oriented in the insulin–receptor complex, may act as a nucleophile interacting with the receptor. Furthermore, hydrogen bonding may alter the conformation of the receptor, thereby participating in the mechanism of action of the hormone. Evidence of conformational change of the receptor on binding with insulin has been presented (Pilch & Czech, 1980; Donner & Yonkers, 1983). The initiation of the action of insulin has been postulated to be related to one or more of at least four events: (1) a proteolytic event leading to the formation of a second messenger (small peptide);[2] (2) stimulation of a selective phospholipase C activity that acts on a glycolipid to yield mediators for some of the actions of insulin (Saltiel et al., 1986); (3) activation of the tyrosine protein kinase activity of the insulin receptor (Kasuga et al., 1982; Roth & Cassell, 1983); (4) disulfide–sulfhydryl exchange with the insulin receptor (Clark & Harrison, 1982). Any of these events could be influenced by the nucleophilic or strong hydrogen bond forming ability of the dipolar form of the A^{20}–A^{21} amide bond.

The idea that peptide bonds of the insulin backbone may play an important role in the physiological action of insulin is not novel. Dodson et al. (1979) have indeed presented evidence and argued convincingly that hydrogen bonding between the main chain oxygens and nitrogens (peptide bonds) of the insulin backbone and complimentary structures of the receptor is implicated in the expression of biological activity. In the two preceding papers (Chu et al., 1987a,b) and in this paper we present evidence that the A^{20}–A^{21} amide moiety may be one of the insulin backbone peptide bonds involved in the expression of the biological activity of this hormone and may play a unique role in this respect.

[2] For a review, see Symposium on Cellular Dynamics of Insulin Action (1982).

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Supplementary Material Available

Complete details of the synthesis of compounds I–VIII and Ia–VIIIa including references (11 pages). Ordering information is given on any current masthead page.

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