
Peptide Interactions of Proinsulin C-Peptide

2

Charlotte Nerelius, Emma Lindahl, Michael Landreh,
and Hans Jörnvall

Keywords

Proinsulin C-peptide • Insulin • Surface plasmon resonance • Mass spectrometry • Gel electrophoresis • Oligomerization • Chaperone • Amyloid-like

Introduction

This review summarizes results on peptide interactions of proinsulin C-peptide. As we see it, knowledge on functional interactions of C-peptide has passed through several stages of research, now with at least three modes of postulated molecular explanations for the beneficial effects of C-peptide in diabetes. Here, we first summarize the stages and then concentrate on the subject of C-peptide interaction studies. The latter should not be interpreted to contradict other modes of action, but rather to reflect our present emphasis and interest.

C-peptide functions in insulin synthesis and secretion. The first era of C-peptide research established the existence of C-peptide, its structure, proforms, cosecretion with insulin into

blood, and its basic function in insulin synthesis and secretion, all largely started and led by Steiner and coworkers [1, 2]. It now constitutes textbook material in biochemistry, endocrinology, and molecular medicine and is a lifetime achievement of great importance to our understanding of diabetes.

C-peptide in receptor-like cellular binding. The second era, derived from the discovery of the C-peptide cosecretion with insulin into blood, initiated studies to find a hormonal role for C-peptide. Johansson, Wahren, and collaborators showed clinically observable effects of C-peptide [3, 4]. They also initiated molecular studies, including those in our laboratory [5]. This phase led to the discovery of a specific cellular binding of C-peptide, sensitive to pertussis toxin, probably related to a G-protein-coupled receptor [6]. The C-terminal pentapeptide of C-peptide was able to compete with this binding, suggesting that this part of C-peptide constitutes an “active site.” The binding constant determined suggested receptor saturation at normal, nanomolar C-peptide concentrations in vivo and a functional loss of this activity in type 1 diabetes. Gradually,

C. Nerelius • E. Lindahl • M. Landreh • H. Jörnvall (✉)
Department of Medical Biochemistry
and Biophysics (MBB), Karolinska Institutet,
SE-171 77, Stockholm, Sweden
e-mail: Hans.Jornvall@ki.se

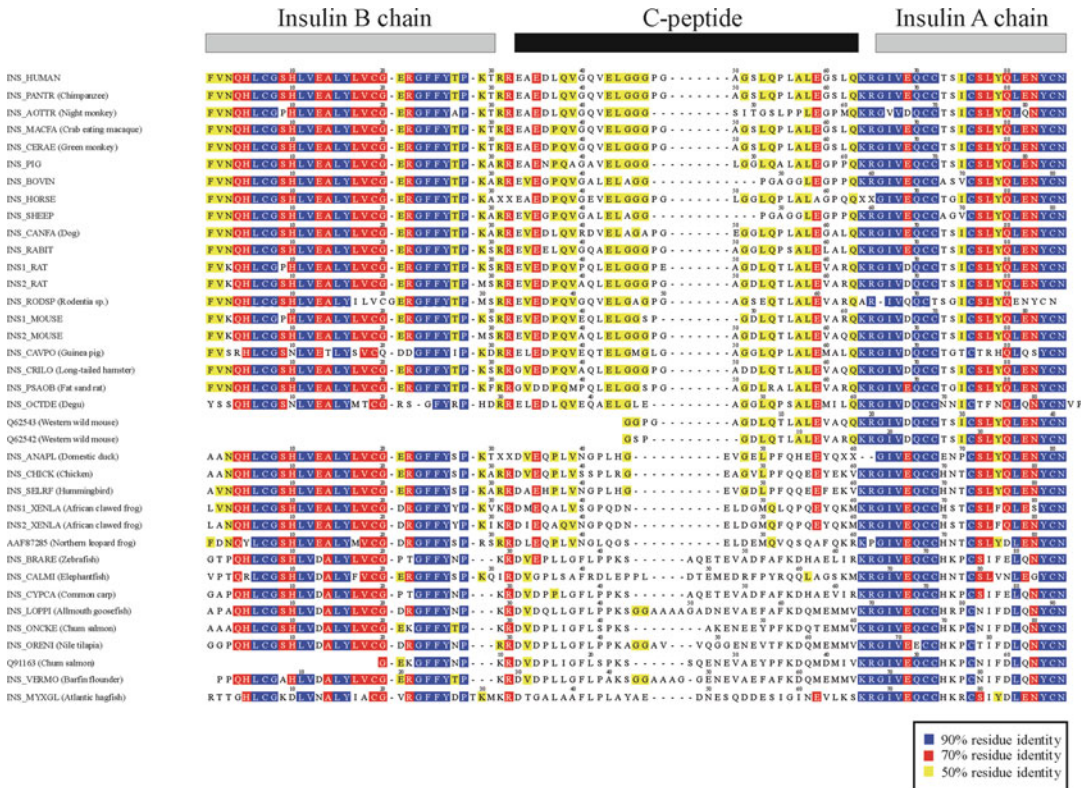


Fig. 2.1 Conservation pattern for the residues of proinsulin in 37 species variants, from the human (*top*) to Atlantic Hagfish (*bottom*). Color code: *Blue*, 90% conservation; *red*, 70%; *yellow*, 50%. As is obvious from the figure, insulin is extremely well-conserved, which is typi-

cal for a peptide hormone in defined receptor interactions and functions, while C-peptide has a completely different pattern, not promoting the concept of tight, conserved C-peptide receptor interactions like for insulin. Original figure from ref. [40] now (reproduced with permission)

this led to the concept of type 1 diabetes being a double-hormone deficiency disorder and its late complications as possibly derived from chronic lack of C-peptide [7]. Additional findings from many groups (reviewed in [7]) supported the concept of a receptor-mediated C-peptide signaling pathway, and clinical studies with C-peptide treatment confirmed beneficial influences on deteriorating functions in type 1 diabetes [8, 9]. However, a receptor-mediated role of C-peptide is still unresolved and does not appear to have caught the attention of the insulin-producing pharmaceutical industry. The enigmas include: (1) a receptor has not been found or characterized; (2) the homology pattern of C-peptide is markedly different from that of insulin (Fig. 2.1) and other traditional signaling peptides; (3) the long time to life-threatening late complications in

diabetes type 1 is at least not typical of the type of immediate withdrawal effects noticed upon lack of insulin and many other signaling peptides; and (4) a definite disease link between the late complications and C-peptide hormonal effects is yet indirect, at most.

C-peptide cellular internalization. The third era showed C-peptide to be rapidly internalized into several cell types [10], and into the nucleoli, where it binds to histones and promotes histone acetylation and rRNA transcription as an intracrine factor via epigenetic mechanisms [11]. This finding opened a new approach and may blur interpretations previously ascribed to receptor-activated signaling, since intracrine effects could also lead to protein inductions. In fact, recent follow-up studies by mRNA microarrays of early

C-peptide influences on renal tubular cells suggest multiple effects on expression levels [12]. This research is complex and requires further work, although our results on C-peptide internalization have been confirmed by others [13] and noticed in the field of intracrine peptides [14].

In other attempts, we found intra- and extracellular proteins binding to Biacore chip-attached C-peptide, as briefly reported [15], and have used a specific interactive labeling to establish an association of C-peptide with yet another protein, protein tyrosine phosphatase [16], earlier implied in insulin-like signaling effects of C-peptide via scaffolding on insulin signaling ([17], reviewed in [18]). In conclusion, C-peptide can interact with several extra- and intracellular proteins. This is not surprising, considering the extreme negative charge of C-peptide, facilitating interactions with other charged proteins in the complexity of tissues.

C-peptide in oligomeric peptide interactions. The early, chip-based screenings for binding interactions also led to a further mode of possible C-peptide peripheral action, via oligomeric peptide binding and its effects on hetero- and homopeptide interactions of C-peptide [19]. This field is reviewed below. We therefore now appear to have three separate modes and localizations of C-peptide interactions, in addition to its role in insulin synthesis and secretion: surface-mediated receptor binding, intracrine effects, and oligomeric peptide interactions. This multiplicity of measurable effects was unexpected of a peptide that was long regarded as having no bioactivity beyond that in insulin synthesis and secretion.

Peptide Interactions

C-peptide desaggregation of insulin oligomers in a chaperone-like manner. In our early attempts with affinity assays in Biacore experiments, we tested for C-peptide interactions with insulin, but never noticed a strong binding between a chip with one of the peptides and an eluent with the other. However, under some conditions binding appeared to be observable in assays with insulin on the chip and insulin plus C-peptide in the eluent. This was interpreted to mean that insulin oligomers first

formed on the chip and then bound C-peptide in an apparently specific manner, since replacement of C-peptide with scrambled C-peptide (i.e., C-peptide with the same composition but random sequence) did not elicit the same binding [19]. It appeared as if the best fit of the binding curves was that of a model where an insulin dimer bound a C-peptide monomer [19]. However, these binding estimates are sensitive to many variables, and their exact stoichiometry should not necessarily be interpreted to be as initially estimated. Nevertheless, the binding analyses indicated that C-peptide/insulin interactions may involve peptide oligomers, at least of insulin, and that specific interactions between C-peptide and insulin may play a role.

This was unexpected and led to further mass spectrometric analyses of solutions with C-peptide, insulin, and insulin/C-peptide mixtures which revealed two findings (Fig. 2.2).

One, mass spectrometry analysis of peptide solutions clearly shows oligomers of both peptides, but with different patterns. The insulin oligomers are easily detectable and are already well-known in the literature [20, 21]. They appeared to reach the hexameric stage, while the C-peptide oligomers were somewhat less abundant and appeared to approach lower stages, like trimers. Initially, we did not fully believe in the C-peptide oligomers and did not emphasize their presence, since C-peptide is highly charged (including six negatively charged residues) and was considered to be self-repulsive.

Two, the oligomers were largely nondetectable in the peptide mixtures, and especially the strong insulin oligomers appeared to vanish upon addition of C-peptide [19, 22]. This suggested that C-peptide disintegrated insulin oligomers, forcing insulin into the monomeric state, thus acting on it in a chaperone-like manner.

These findings initiated further in vivo studies of diabetic patients receiving C-peptide in addition to their regular insulin injections [19]. Simultaneous injection of insulin and C-peptide was then found to lead to a more rapid increase in plasma insulin, suggesting that C-peptide also in vivo has an insulin-desaggregating effect (Fig. 2.3 [19]). Thus, our concept of a chaperoning effect of C-peptide on insulin oligomers appeared to be valid also in vivo. In fact, this

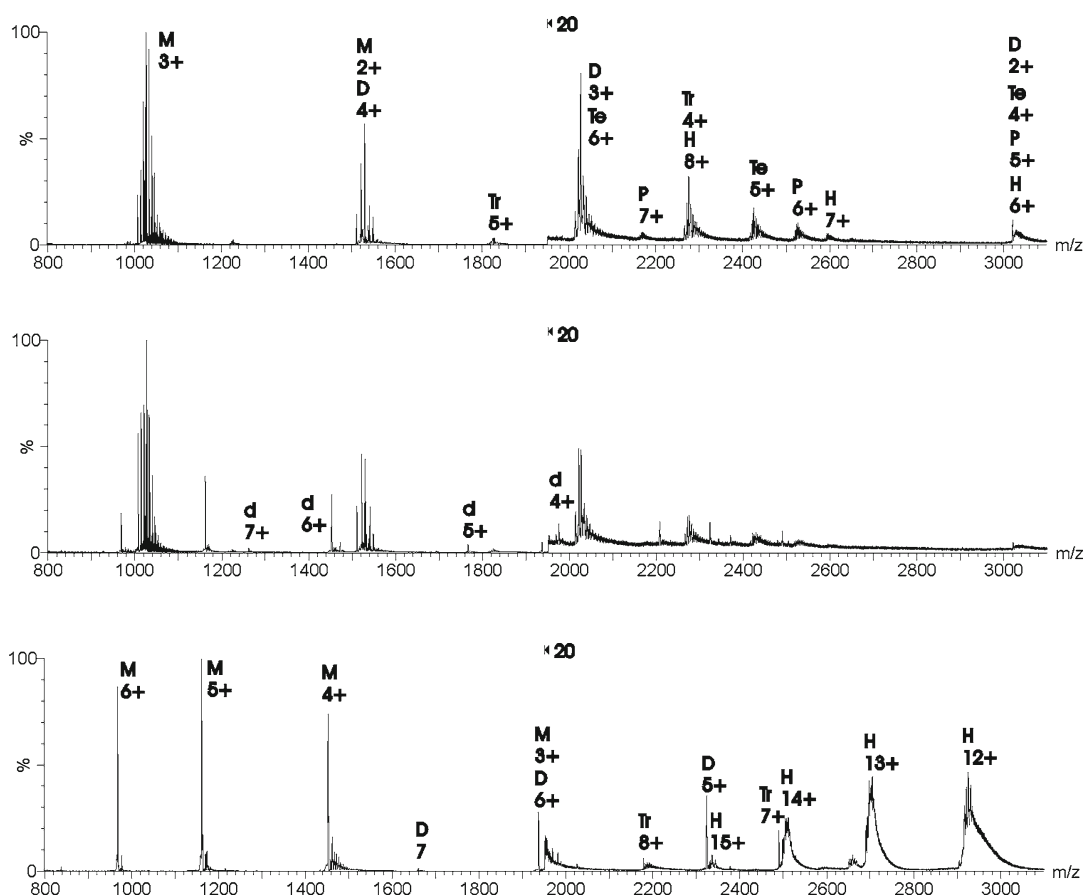


Fig. 2.2 Mass spectra centring on the part showing the maximal oligomers, with only C-peptide (*top*) and only insulin (*bottom*), but disaggregated oligomers in the

C-peptide/insulin mixture (*middle*). Original figure from ref. [22] now (reproduced with permission)

direct link between insulin plasma increase and C-peptide injection still appears to be a direct link between a clinically observable effect of C-peptide and the observed molecular interaction.

Oligomer formations of C-peptide itself. The findings regarding insulin-C-peptide interactions led us to further analyses of the possible oligomeric nature of C-peptide itself by additional mass spectrometric and gel electrophoretic studies.

Regarding the mass spectrometric analyses, we confirmed the previously not emphasized observation that C-peptide itself is in equilibrium with a family of homooligomers and is observable by electrospray analysis [22]. It showed that much like C-peptide disaggregates insulin oligomers, insulin has a similar effect on C-peptide

oligomers (Fig. 2.2), either through desoligomerization or because C-peptide interaction with insulin is stronger than C-peptide homooligomerization. In any event, both insulin and C-peptide alone exhibit an oligomerization tendency. Similarly, gel electrophoretic analyses also established the ability of C-peptide to form oligomers in many solutions [22, 23]. This was initially observed when we set up Western blots of C-peptide in order to detect the peptide in cellular internalization studies [10]. The combined results from mass spectrometry and gel electrophoresis made us draw several conclusions regarding the interactions of C-peptide and insulin oligomers.

Homooligomers. The two peptides can individually form homooligomers in solution, discernable

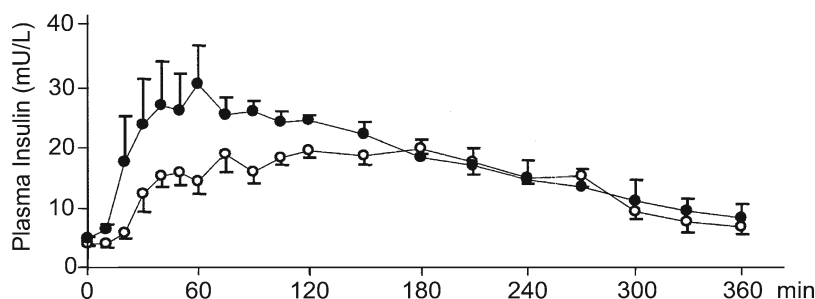


Fig. 2.3 Plasma insulin after injection of insulin (unfilled circles) or the same amount insulin with C-peptide also added (filled circles). As shown, the latter increases blood glucose to higher levels and thus a more rapid early phase,

compatible with C-peptide desaggregation of insulin oligomers in vivo in diabetic patients. Original figure from ref. [19] now (reproduced with permission)

with several methods. Monomers are by far the major components, at least in fresh solutions, and the oligomeric patterns differ, involving several types of multiplicity, but with hexamers being strong oligomers of insulin and trimers of C-peptide. Simple monitoring showed concentration to be important, and less intense mass spectrometric oligomer peaks were observed upon dilution. However, the relative patterns between different oligomeric components appeared unaffected by dilution, with similar peak ratios for 300 and 30 μ M C-peptide at weakly acidic conditions [22]. Upon maximal amplification, oligomers could be mass spectrometrically detectable in the sub- μ M range. Although pH had an effect (oligomers were not detectable in nanospray analysis of weakly basic solutions), the patterns visible with sufficient amplification in the electrospray analyses appeared to have surprisingly similar peak ratios upon spraying from pH 5 (with weak HCl) and pH 8 (with 10 mM ammonium bicarbonate [22]). Temperature, time, and metal ions all increased the C-peptide oligomerization tendency. We concluded that C-peptide in solution is by far largely monomeric, but can form an equilibrium with oligomers present in low yield.

Heterointeractions. The two peptides can interact. Heterooligomers are mass spectrometrically noticeable in the peptide mixtures, but to a low extent, below that of the homooligomers. The major homooligomers of either peptide literally vanish with the presence of also the other peptide (Fig. 2.2) [19, 22]. Hence, monomerization

especially of insulin is promoted in the mixture, which is consistent with the interpretation of the rapid plasma rise upon coinjection in diabetic patients (Fig. 2.3) [19].

Reaction-like transitions. Combined, these two observations suggest a complex pattern for the heterointeractions between the two peptides. Obviously, they must first bind each other in order to interact, causing the surface plasmon resonance binding signals. However, binding is apparently transient, causing an effect (desoligomerization). The apparent reaction resembles chaperone-like actions, where desaggregation results and where interactions involve polymers. Hence, C-peptide appears to have a chaperone-like activity for oligomerized/aggregated insulin.

Aggregation and possible fibrillation of C-peptide. Continuing the interactive studies, an obvious next step was to evaluate to what extent C-peptide might oligomerize further, polymerize, aggregate, and even perhaps fibrillate, having amyloid-like properties.

Already gel electrophoretic analyses revealed the presence of higher aggregates (Fig. 2.4 [22]), and with time, positive staining with thioflavin T [22], which is often considered indicative of fibrillat β -sheet structures. Together with Lind et al. [23], the behavior of C-peptide in the presence of sodium dodecyl sulfate (SDS) as a catalyst for structural transitions was therefore monitored. It then became obvious that C-peptide can form large oligomers and aggregates in

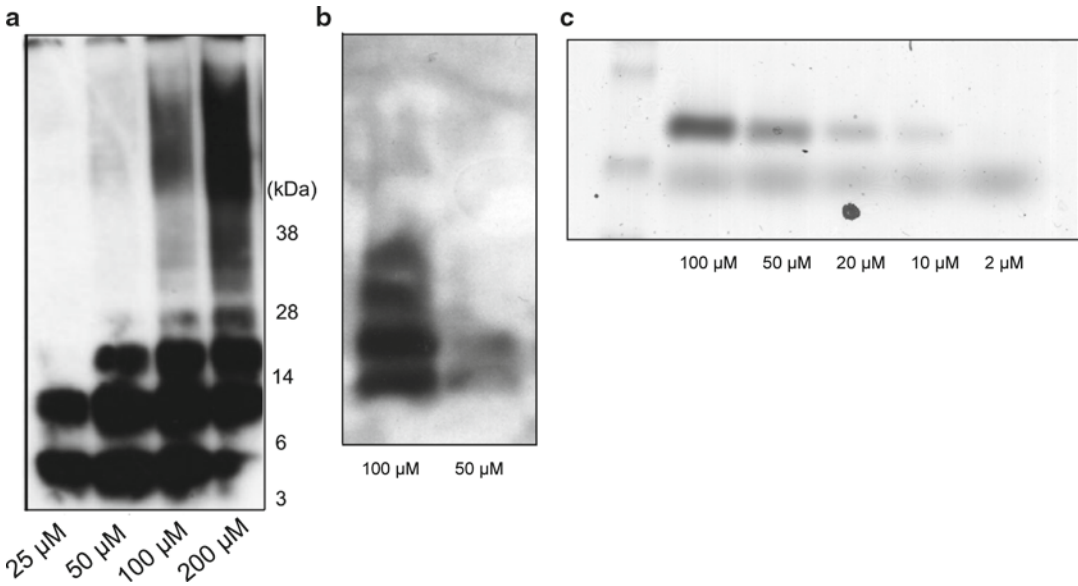


Fig. 2.4 C-peptide oligomer formations as observed in nondenaturing gel electrophoresis and Western blotting (a), denaturing gel electrophoresis, blotting, and double

antibody detection (b) or direct photography of rhodamine-labeled C-peptide (c). Original figure from ref. [22] now (reproduced with permission)

complex transitions that can be monitored by NMR spectroscopy and dynamic light scattering. Monomers were initially the major component, but with time and increasing concentrations, C-peptide became stainable with thioflavin T [23] in a manner resembling that observed for amyloidogenic proteins [24].

We therefore concluded that C-peptide is capable of oligomerization in multiple steps under suitable conditions, and that this can be modeled in the presence of SDS. The end result can apparently lead to aggregation and amyloid-like, presumably β -sheet aggregates. Therefore, solutions of C-peptide, like those of insulin, appear to be in equilibrium with a small population of oligomers that potentially can aggregate further. In the early stages of this equilibrium pathway, C-peptide can in addition interact with oligomeric insulin and desaggregate such oligomers. The two peptides therefore have a complicated biophysical behavior, which suggests that both their absolute amounts and ratios may be of importance. Undoubtedly, this whole series of observations opens one further mode of possible pathways in which C-peptide could constitute a bioactive

peptide. If the amyloid-like properties turn out to be of importance, links to depository diseases and other conditions may also apply. As initially noted here, we therefore now know of three modes of postsecretion, C-peptide activity, and possible role in diabetes: through receptor-mediated cell surface pathways, intracrine pathways, and peptide interaction pathways. Before trying to evaluate which of these activities may explain the observed beneficial effects of C-peptide replacement in type 1 diabetes, it appears motivated to reflect on the possible relevance of peptide interactions in vivo.

Precaution. We are aware that objections can be raised against attempts at in vivo conclusions regarding consequences of the C-peptide oligomerizations. First, most of the biophysical studies were performed at nonphysiological peptide concentrations and nonphysiological environmental conditions. In vivo, SDS does not occur, circulating C-peptide is present at nM concentrations, and insulin is present at a considerably lower concentration. Before dismissing the peptide results on such objections, however, one should consider also the following facts.

First, SDS is not necessary for the observed effects. The gel patterns (Fig. 2.4) and the mass spectrometry (Fig. 2.2) show oligomerizations also in nonextreme, aqueous solutions and surface plasmon resonance studies suggest binding of peptide oligomers in ordinary solvents. SDS in the biophysical study [23] was used as a catalyst to promote transitions, much like trifluoroethanol is frequently used in NMR spectroscopy studies to promote detection of helix tendencies.

Second, the very nature of the structural transitions to β -sheet fibril conformation, as in the already known amyloidogenic diseases, is profound and involves large rearrangements of the proteins involved. Hence, rough tools for study of these phenomena need not be irrelevant.

Third, regarding peptide concentrations, maximal amplification in the mass spectrometric studies detected oligomers down to sub- μ M C-peptide levels [22]. In the prediabetic type 2 diabetes stage, there is hyperpeptidism, as well as locally at injection sites in type 1 diabetics where deposits have been found [25], making the span between the in vivo and experimental conditions about two orders of magnitude for C-peptide, a range that is often considered sufficient for chemical cross-interpretations. Initial transitions are in apparent equilibrium, which makes rapid shifts towards further transitions possible, should the conditions change.

In conclusion, the objections suggested above constitute only relative concerns, making further proof for a link between in vitro observed oligomerizations and in vivo diabetic disease desirable. Hence, these objections are not excluding a relevance of peptide interactions in vivo, especially not under special conditions such as hyperpeptidism or peptide ratio imbalances.

A second precaution concerns the fact that biophysical phenomena are complex and difficult to study; mass spectrometry involves transitions to gas-phase measurements and surface plasmon resonance surface attachments and model curve fittings. Additional studies are therefore desirable. However, the present agreements between interpretations from mass spectra, surface plasmon resonance, and gel electrophoresis appear relevant and are known to be so in many other systems.

A third precaution concerns the link between C-peptide molecular effects and observed beneficial clinical effects. Before dismissal of peptide interactions on that issue, one should consider also the following two facts.

There is, as mentioned above, a direct experimental result that indicates a link between peptide interactions and clinical effects. Thus, in vivo coinjection of insulin and C-peptide appears to enhance the plasma insulin level obtained [19]. It is hard to visualize any other mechanism to explain that observable curve (Fig. 2.3) than direct peptide interaction. We initially probably underestimated the strength of this fact and should have emphasized it further.

The only concern with the depository model that is difficult to answer at present is which deposit and where that may be critical for diabetes. Reports exist of deposits of several peptides (including of insulin [26] and C-peptide [27]), but they still appear few and difficult to consistently interpret regarding size and organ. Perhaps, though, microdeposits or deposits in special organs, like the pancreas, anyway loaded with insulin, C-peptide, and other peptides, may have escaped detection or proper attention.

In conclusion, considering the clinical beneficial effects of C-peptide, which to us seems reasonable, based on the amelioration of kidney and nerve dysfunctions, organs commonly affected in diabetes, all three hypotheses on how the molecular mechanisms may work appear feasible. None can be excluded, but the peptide interactions as explanation appear equally valid or even more so than the other alternatives at the present stage.

Perspectives and Future Work

With the precautions and reservations thus considered, which are the most attractive consequences of homo- and heteropeptide interactions as a molecular explanation for the clinical effects of C-peptide?

- First, of the several attractive consequences, two enigmas of C-peptide actions (the lack of strict sequence conservation and the slow onset of type 1 complications), which constitute problems

with the receptor-mediated explanation, are no problems with the peptide interactive model. A slow onset of the chronic complications could be consistent with the depository model, thus suggesting that amyloid-like properties of insulin would be a factor in the late complications of type 1 diabetes. Notably, the only drug all type 1 diabetics have got for decades is insulin, so if the insulin desaggregation power of C-peptide is of relevance, the implication is that insulin is its target in the beneficial effect on the late complications. Similarly, the lack of C-peptide sequence conservation would be less important in the peptide interactive model, since charge (which is conserved in C-peptide) rather than sequence is important in oligomerization [23]. Indeed, relationships have been demonstrated also between diabetes and Alzheimer's disease [28–30].

- Second, the peptide interaction model would also be consistent with the onset of type 2 diabetes, in which increased levels of insulin and/or C-peptide rather than hyperglycemia have been claimed as disease-promoting factors [31, 32]. Hence, not only would type 1 late complications, but also those of type 2 diabetes onset be consistent with the peptide interactive model. The implication is then that in type 2 diabetes, it is the absolute amount of peptides rather than their ratio (as in type 1 diabetes) that is wrong and that the depository tendency would derive from either of insulin, C-peptide, or other peptides, alone or in mixtures.
- Third, also metabolically, a depository tendency of C-peptide and insulin could fit known facts. Thus, peptide deposits in general may derive not only from increased synthesis, but also from decreased breakdown. In this regard, it is of interest that insulin and C-peptide appear to depend on similar enzyme activities for degradation [33]. This enzyme activity, in the case of insulin-degrading enzyme, is sensitive to inhibition by alkylation of a critical Cys residue through lipid peroxidation products [34], hence compatible with the fact that increased abdominal fat is a risk factor for type 2 diabetes [35].

- Finally, there is a tantalizing theoretical parallel in the model of C-peptide as a chaperone for reversal of insulin depository tendencies, in the fact that several depository diseases recently have been ascribed to peptide deposits derived from proforms biosynthetically equipped with an autospecific chaperone (a BRICHOS domain protein [36]). Lung surfactant peptide C is such a depository peptide derived from a proform (prosurfactant protein C) equipped with a C-terminal autospecific chaperone [37, 38]. Hence, nature has apparently not evolutionarily eliminated these structures although potentially dangerous, but may equip their proforms with a “rucksack” of a specific chaperone reducing the depository tendency. Perhaps, proinsulin is another such type of proform, with an inbuilt C-peptide “rucksack” for the active hormone function of insulin. Hence, both types of diabetes may reflect imbalances, where the insulin/C-peptide pair illustrates general principles for regulatory peptides in nature.

The question then arises, how do we proceed to expand our knowledge as to how C-peptide ameliorates diabetic renal and neural disorders? Of course, one mode of approach is to continue investigations of all three molecular explanations, including a search for specific deposits in the endocrine pancreas, nerves, and kidneys, which are the organs ultimately affected. However, such broad approaches may be difficult, and our latest results appear to offer a further opening.

Thus, using C-peptide fragments we recently found additional evidence for particular fragments of C-peptide having separate activities in relation to the three molecular explanation modes. The N-terminal fragment (residues 1–11) appears to be ascribable to an insulin interaction of C-peptide and is not identical to the site for the homo-C-peptide oligomerization [39]. Previously, we have found the C-terminal pentapeptide of C-peptide to be associated with the receptor-mediated actions [5, 6], and the whole C-peptide to be tripartite (Fig. 2.5 [40]). Hence, further work with definitions of the C-peptide fragment activities may define shorter segments with just one activity rather than the intact C-peptide with all three

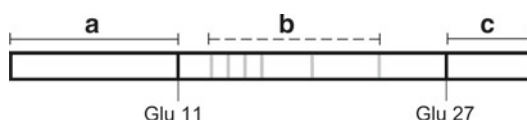


Fig. 2.5 Schematic representation of C-peptide into a tripartite correlation with different properties. (a) the N-terminal segment with Glu11 (black vertical line) apparently important in insulin binding [39]; (c) the C-terminal part with Glu27 (black vertical line) ascribed importance in the receptor-mediated capacity [5, 6], and (b) the intervening part with many Gly and Pro (gray vertical lines) and resulting separate structural properties, possibly a part of the homo-oligomerizations [39]

activities. If so, C-peptide fragments or analogs with just one of the three alternative effects may allow for treatment with only the beneficial part, eliminating even the possibility of a risk of depository tendencies derived from other segments.

In summary, recent results have given further complexity and unexpected multiplicity in functional interpretations of C-peptide molecular interactions, but also offer an approach to continue with fragment research that may finally give a both efficient (chaperone-like) and in all circumstances safe (nondepository) C-peptide derivative.

Acknowledgments We thank John Wahren, Jan Johansson, Jawed Shafqat, Lena Mäler, Michael Henriksson, Ermas Melles, Juan Astorga-Wells, Gunvor Alvelius, and coworkers in several other laboratories for valuable collaborative efforts. Grant support from the Swedish Research Council (03X-3532) and the Knut and Alice Wallenberg Foundation is gratefully acknowledged.

References

1. Steiner DF, Oyer PE. The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. *Proc Natl Acad Sci U S A*. 1967;57:473–80.
2. Steiner DF, Cunningham D, Spigelman L, et al. Insulin biosynthesis: evidence for a precursor. *Science*. 1967;157:697–700.
3. Johansson BL, Sjöberg S, Wahren J. The influence of human C-peptide on renal function and glucose utilization in type1 (insulin-dependent) diabetic patients. *Diabetologia*. 1992;35:121–8.
4. Johansson BL, Linde B, Wahren J. Effects of C-peptide on blood flow, capillary diffusion capacity and glucose utilization in the exercising forearm of type1 (insulin-dependent) diabetic patients. *Diabetologia*. 1992;35:1151–8.

5. Ohtomo Y, Bergman T, Johansson B-L, et al. Differential effects of proinsulin C-peptide on Na⁺, K⁺-ATPase activity of renal tubule segments. *Diabetologia*. 1998;41:287–91.
6. Rigler R, Pramanik A, Jonasson P, et al. Specific binding of proinsulin C-peptide to human cell membranes. *Proc Natl Acad Sci U S A*. 1999;96:13318–23.
7. Wahren J, Ekberg K, Jörmvall H. C-peptide is a bioactive peptide. *Diabetologia*. 2007;50:503–9.
8. Ekberg K, Brismar T, Johansson B-L, et al. C-peptide replacement therapy and sensory nerve function in type 1 diabetes neuropathy. *Diabetes Care*. 2007;30:71–6.
9. Ekberg K, Johansson B-L. Effect of C-peptide in diabetic neuropathy in patients with type 1 diabetes. *Exp Diabetes Res*. 2008;2008:457912.
10. Lindahl E, Nyman U, Melles E, et al. Cellular internalization of proinsulin C-peptide. *Cell Mol Life Sci*. 2007;64:479–86.
11. Lindahl E, Nyman U, Zaman F, et al. Proinsulin C-peptide regulates ribosomal RNA expression. *J Biol Chem*. 2010;285:3462–9.
12. Lindahl E, Nordquist L, Müller P, et al. Early transcriptional regulation by C-peptide in freshly isolated rat proximal tubular cells. *Diabetes Metab Res Rev*. 2011, epub doi:10.1002/dmrr.1220.
13. Luppi P, Geng X, Cifarelli V, et al. C-peptide is internalised in human endothelial and vascular smooth muscle cells via endosomes. *Diabetologia*. 2009;52:2218–28.
14. Re RN, Cook JL. Mechanisms of disease: intracrine physiology in the cardiovascular system. *Nat Clin Pract Cardiovasc Med*. 2007;4:549–57.
15. Shafqat J, Melles E, Wiberg D, et al. Proinsulin C-peptide elicits disaggregation of insulin resulting in enhanced physiological insulin effects. In: Melles E. C-peptide structural and functional relationships studied by biosensor technology and mass spectrometry. Thesis, Karolinska Institutet, Stockholm, Sweden; 2005.
16. Jägerbrink T, Lindahl E, Shafqat J, et al. Proinsulin C-peptide interaction with protein tyrosine phosphatase 1B demonstrated with a labeling reaction. *Biochem Biophys Res Commun*. 2009;387:31–5.
17. Li Z-G, Qiang X, Sima AAF, et al. C-peptide attenuates protein tyrosine phosphatase activity and enhances glycogen synthesis in L6 myoblasts. *Biochem Biophys Res Commun*. 2001;280:615–9.
18. Sima AAF. Diabetic encephalopathies – the emerging complications. *Acta Diabetol*. 2010;47:279–93.
19. Shafqat J, Melles E, Sigmundsson K, et al. Proinsulin C-peptide elicits disaggregation of insulin resulting in enhanced physiological insulin effects. *Cell Mol Life Sci*. 2006;63:1805–11.
20. Brange J, Andersen L, Laursen ED, et al. Towards understanding insulin fibrillation. *J Pharm Sci*. 1997;86:517–25.
21. Nettleton EJ, Tito P, Sunde M, et al. Characterization of the oligomeric states of insulin in self-assembly and amyloid fibril formation by mass spectrometry. *Biophys J*. 2000;79:1053–65.

22. Jörnvall H, Lindahl E, Astorga-Wells J, et al. Oligomerization and insulin interactions of proinsulin C-peptide: threefold relationships to properties of insulin. *Biochem Biophys Res Commun.* 2010;391:1561–6.
23. Lind J, Lindahl E, Perálvarez-Marín A, et al. Structural features of proinsulin C-peptide oligomeric and amyloid states. *FEBS J.* 2010;277:3759–68.
24. Khurana R, Coleman C, Ionescu-Zanetti C, et al. Mechanism of thioflavin T binding to amyloid fibrils. *J Struct Biol.* 2005;151:229–38.
25. Dische FE, Wernstedt C, Westermark GT, et al. Insulin as an amyloid-fibril protein at sites of repeated insulin injections in a diabetic patient. *Diabetologia.* 1988;31:158–61.
26. Furuta T, Seino J, Saito T, et al. Insulin deposits in membranous nephropathy associated with diabetes mellitus. *Clin Nephrol.* 1992;37:65–9.
27. Marx N, Walcher D, Raichle C. C-peptide colocalizes with macrophages in early arteriosclerotic lesions of diabetic subjects and induces monocyte chemotaxis in vitro. *Arterioscler Thromb Vasc Biol.* 2004;24:540–5.
28. Janson J, Laedtke T, Parisi JE, et al. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes.* 2004;53:474–81.
29. Li Z-G, Zhang W, Sima AAF. Alzheimer-like changes in rat models of spontaneous diabetes. *Diabetes.* 2007;56:1817–24.
30. Sima AAF, Li Z-G. Diabetes and Alzheimer's disease – is there a connection? *Rev Diabet Stud.* 2007;4:161–8.
31. Rosselin G, Rathery M, Assan R, et al. Hyperinsulinism in moderate diabetes and prediabetes. *Rein Foie.* 1966;9:47–62.
32. Park SH, Marso SP, Zhou Z, et al. Neointimal hyperplasia after arterial injury is increased in a rat model of non-insulin-dependent diabetes mellitus. *Circulation.* 2001;104:815–9.
33. Melles E, Jörnvall H, Tryggvason S, et al. Degradation of proinsulin C-peptide in kidney and placenta extracts by a specific endoprotease activity. *Cell Mol Life Sci.* 2004;61:2979–82.
34. Neant-Fery M, Garcia-Ordóñez RD, Logan TP, et al. Molecular basis for the thiol sensitivity of insulin-degrading enzyme. *Proc Natl Acad Sci U S A.* 2008;105:9582–7.
35. Carey DG, Jenkins AB, Campbell LV, et al. Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes.* 1996;45:633–8.
36. Sánchez-Pulido L, Devos D, Valencia A. BRICHOS: a conserved domain in proteins associated with dementia, respiratory distress and cancer. *Trends Biochem Sci.* 2002;27:329–32.
37. Nerelius C, Gustafsson M, Nordling K, et al. Anti-amyloid activity of the C-terminal domain of proSP-C against amyloid β -peptide and medin. *Biochemistry.* 2009;48:3778–86.
38. Fitzen M, Alvelius G, Nordling K, et al. Peptide binding specificity of the prosurfactant protein C Brichos domain analyzed by electrospray mass spectrometry. *Rapid Commun Mass Spectrom.* 2009;23:3591–8.
39. Nerelius C, Alvelius G, Jörnvall H. N-terminal segment of proinsulin C-peptide active in insulin interaction/desaggregation. *Biochem Biophys Res Commun.* 2010;403:462–7.
40. Henriksson M, Nordling E, Melles E, et al. Separate functional features of proinsulin C-peptide. *Cell Mol Life Sci.* 2005;62:1772–8.

Diabetes & C-Peptide

Scientific and Clinical Aspects

Sima, A.A.F. (Ed.)

2012, XIII, 169 p. 63 illus., 35 illus. in color., Hardcover

ISBN: 978-1-61779-390-5

A product of Humana Press