The Conformation of Glucagon: Predictions and Consequences†

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ABSTRACT: It is proposed that glucagon, a polypeptide hormone, is delicately balanced between two major conformational states. Utilizing a new predictive model [Chou, P. Y., and Fasman, G. D. (1974), *Biochemistry 13,* 222] which considers all the conformational states in proteins (helix, β sheet, random coil, and β turns), the secondary structural regions of glucagon are computed herein. The conformational sensitivity of glucagon may be due to residues 19–27 which have both α-helical potential ($\langle P_\alpha \rangle$ = 1.19) as well as β-sheet potential ($\langle P_\beta \rangle$ = 1.25). Two conformational states are predicted for glucagon. In predicted form (a), residues 5–10 form a β-sheet region while residues 19–27 form an α-helical region (31% α, 21% β) agreeing well with the circular dichroism (CD) spectra of glucagon. The similarity in the CD spectra of glucagon and insulin further suggests the presence of β structure in glucagon, since X-ray analysis of insulin showed 24% β sheet. In predicted form (b), both regions, residues 5–10 and residues 19–27, are β sheets sheets (0% α, 52% β) in agreement with the infrared spectral evidence that glucagon gels and fibrils have a predominant β-sheet conformation. Since three reverse β turns are predicted at residues 2–5, 10–13, and 15–18, glucagon may possess tertiary structure in agreement with viscosity and tritium–hydrogen exchange experiments. A proposal is offered concerning an induced $\alpha \rightarrow \beta$ transition at residues 22–27 in glucagon during receptor site binding. Amino acid substitutions are proposed which should disrupt the β sheets of glucagon with concomitant loss of biological activity. The experimental findings that glucagon aggregates to form dimers, trimers, and hexamers can be explained in terms of β-sheet interactions as outlined in the present predictive model. Thus the conflicting conclusions of previous workers, concerning the conformation of glucagon in different environments, can be rationalized by the suggested conformational transition occurring within the molecule.

Glucagon is a 29 amino acid polypeptide hormone (mol wt 3500) which was discovered 50 years ago (Murlin et al., 1923). Although its amino acid sequence (Figure 1) (identical in pig, bovine, and human) is well established (Bromer et al., 1957, 1971; Thomsen et al., 1972) and much is known about its molecular physiology (Lefebvre and Unger, 1972), its conformational status still remains controversial. Optical rotatory dispersion (ORD) (Blanchard and King, 1966) and circular dichroism (CD) studies (Srere and Brooks, 1969) of glucagon in dilute aqueous solutions from pH 2 to 10 (<1 mg/ml) revealed predominantly a random coil conformation with at most 15% α helix. However, the helicity can be increased to 35% with increased glucagon concentration (10 mg/ml) at pH 10 and to over 50% in 2-chloroethanol (Srere and Brooks, 1969; Gratzer and Beaven, 1969). In the crystalline state, the helical conformation was proposed for glucagon (King, 1965; Haugen and Lipscomb, 1969), but in the gel and fibril forms, the hormone was assumed to be predominantly a β-pleated sheet structure (Gratzer et al., 1967, 1968; Beaven et al., 1969; Epand, 1971). Laser Raman spectral studies show that glucagon undergoes a helix → coil → β-sheet conformational transition from the crystal → aqueous solution → gel state, and that the β conformation is maintained for glucagon gels in the solid state (Yu and Liu, 1972). Recently, on the basis of viscosity, hydrogen–tritium exchange, and CD studies, evidence suggests that glucagon exists in a compact globular conformation in solution (Epand, 1971, 1972a,b; McBride-Warren and Epand, 1972) although thermal difference spectral studies did not reveal any tertiary unfolding process in glucagon (Gratzer et al., 1968). Several models have been proposed for glucagon aggregation in solution; these include a monomer–trimer equilibrium (Blanchard and King, 1966; Gratzer and Beaven, 1969; Gratzer et al., 1972) as well as a monomer–dimer–hexamer association mechanism (Swann and Hammes, 1969).

In the previous predictions of glucagon conformation (Schiffer and Edmundson, 1970; Low et al., 1968, 1971) only the α helix and coil states were considered. Recently Chou and Fasman (1974a,b) have determined the conformational parameters for the 20 amino acids in the helix, β sheet, random coil, and reverse β-turn regions in proteins and have formulated a new predictive model which is capable of elucidating protein secondary structures with 80% accuracy. In particular, they were successful in locating all the helical and β-sheet regions of insulin and pancreatic trypsin inhibitor, and correctly identified the conformational state (α, β, coil) of all residues in these two small proteins with 90% and 86% accuracy, respectively. It is the purpose of this paper to utilize this method to compute the secondary structures (i.e., solid, solution, etc.) of glucagon. The present analyses will assist in explaining some of the interpretative discrepancies of experimental results existing in the literature concerning the behavior of glucagon.

Methods

Applying the newly formulated protein predictive model of Chou and Fasman (1974b) a complete analysis of gluca-

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[1] Abbreviations used are: α, β, c, t represents the α-helical, β-sheet, random coil, and β-turn conformation.

	1	2	3	4	5	6	7	8	9	10	11
	(His)	Ser	(*Gln*)	Gly	*Thr*	(*Phe*)	*Thr*	Ser	Asp	*Tyr*	Ser
α	h	i	h	B	i	h	i	i	i	b	i
β	b	b	h	i	[h	h	h	b	i	h]	b

	12	13	14	15	16	17	18	19	20	21	22
	Lys	*Tyr*	(*Leu*)	Asp	Ser	Arg	Arg	(Ala)	(*Gln*)	Asp	(*Phe*)
α	I	b	H	i	i	i	i	[H	h	i	h
β	b	h	h	i	b	i	i	[I	h	i	h

	23	24	25	26	27	28	29
	(*Val*)	(*Gln*)	(*Trp*)	(*Leu*)	(*Met*)	Asn	*Thr*
α	h	h	h	H	h]	b	i
β	H	h	h	h	H]	b	h

FIGURE 1: Predictive analysis of helical and β-sheet regions in glucagon. Assignments in the first row under each residue refer to helical potential (α), and in the second row to β-sheet potential (β) as defined by Chou and Fasman (1974b). Helical and β residues are also enclosed in parentheses and italicized respectively. The predicted α and β regions are enclosed in brackets. Amino acid sequence as determined by Bromer et al. (1957).

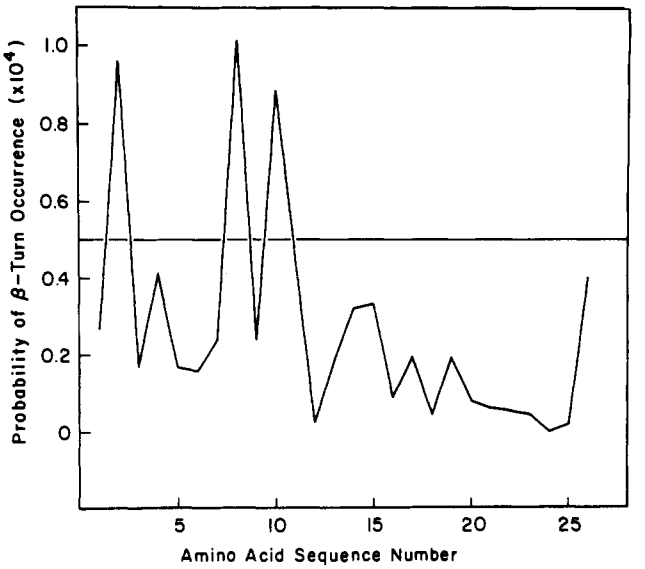

FIGURE 2: Probability that a tetrapeptide β turn begins at site j of glucagon. The horizontal line corresponds to an arbitrary cut-off value of 0.5×10^{-4}. The sharp peaks at j = 2, 8, and 10 indicate the positions of probable chain reversals in glucagon.

gon conformation is given in Figure 1. The assignments of helical (α) and β-sheet potential (β) are given under each residue: H_α (strong helix former), h_α (helix former), I_α (weak helix former), i_α (helix indifferent), b_α (helix breaker), and B_α (strong helix breaker). Replacing the subscript α by β gives the respective β-sheet potential assignments H_β, h_β, I_β, i_β, b_β, and B_β. Helical (h_α and H_α) and β-forming (h_β and H_β) residues are also enclosed in parentheses and italicized, respectively in Figure 1, giving a quick glimpse of helical and β-sheet nucleation centers. These nucleation centers are determined by the predictive method (Chou and Fasman, 1974b) which states: when four helix formers out of six residues or three β formers out of five residues are found clustered together in any native protein segment, the nucleation of these secondary structures begins and propagates in *both* directions until terminated by tetrapeptide breakers with 50% or more helix (or β sheet) breaking or indifferent residues.

The only region of glucagon with a clustering of more than four helical residues is 19–27 where there are eight helical residues out of nine residues with an average helical potential of $\langle P_\alpha \rangle = 1.19$. The tetrapeptide 15–18 $(i_4)_\alpha$ is a helix breaker and Arg-17 and Arg-18 were not included as α because positively charged residues prefer the C-terminal rather than the N-terminal helix (Chou and Fasman, 1974a). The region containing residues 19–27 also contains seven β formers out of nine residues with an average β-sheet potential of $\langle P_\beta \rangle = 1.25$, and is preceded by the tetrapeptide 15–18 $(ibii)_\beta$ which serves as a β-sheet breaker. Since $\langle P_\beta \rangle > \langle P_\alpha \rangle$ for residues 19–27 this region is predicted as β sheet. Another clustering of β residues between residues 5 and 10 (four out of six residues are β forming) is terminated at both ends by the β breakers $(bbhi)_\beta$ at 1–4 and $(bbhh)_\beta$ at 11–14 thus defining the second β region, 5–10, where $\langle P_\beta \rangle = 1.08$ is greater than $\langle P_\alpha \rangle = 0.86$.

The relative probability that a tetrapeptide will form a β turn is $p_t = (f_i)(f_{i+1})(f_{i+2})(f_{i+3})$ where f_i, f_{i+1}, f_{i+2}, and f_{i+3} are respectively the frequency of occurrence for a certain residue at the 1st, 2nd, 3rd, and 4th position of a β turn. In a survey of 12 proteins, Chou and Fasman (1974b) found that the average probability for any tetrapeptide to be in the β turn is $p_t = 0.24 \times 10^{-4}$ and $p_t = 0.5 \times 10^{-4}$ was chosen as a reasonable cut-off value in predicting β turns. Using the f_i, f_{i+1}, f_{i+2}, and f_{i+3} values from Table VII of Chou and Fasman (1974b), the p_t values were computed for the 26 possible tetrapeptide combinations of glucagon and plotted in Figure 2. As can be seen, there are three sites, at residues 2, 8, and 10, with β-turn probability greater than 0.5×10^{-4}. Since region 5–10 was already predicted as β sheet, the tetrapeptides 2–5 and 10–13 were selected for β-turn formation. Although tetrapeptide 15–18 with $p_t = 0.33 \times 10^{-4}$ is below the cut-off point, it is still above the average $p_t = 0.24 \times 10^{-4}$ value for β turns. Since the average β-turn potential for tetrapeptide 15–18, $\langle P_t \rangle = 1.21$, is greater than its average α and β potential ($\langle P_\alpha \rangle = 0.84$, $\langle P_\beta \rangle = 0.83$), it is also predicted as a β turn.

Results and Discussion

The helical, β-sheet, random coil, and β-turn regions of glucagon computed by the above method are summarized in Table I. As can be seen, regions which were not predicted as either α or β are classified in the random coil conformation (c), and have both $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values less than unity. The three predicted β turns, 2–5, 10–13, and 15–18, all have $\langle P_\alpha \rangle < \langle P_t \rangle > \langle P_\beta \rangle$, and appear as terminators of the predicted β-sheet regions 5–10 and 19–27. Although region 19–27 was predicted as β sheet ($\langle P_\beta \rangle = 1.25 > \langle P_\alpha \rangle = 1.19$), there is considerable helical potential in this region as evidenced by the $\langle P_\alpha \rangle = 1.19$ value which is much greater than unity. In fact for residues 19–26, $\langle P_\alpha \rangle = \langle P_\beta \rangle = 1.19$, so that the α and β potentials for this region are identical. Hence both conformations of glucagon are represented in the schematic diagrams of Figure 3. In conformation (a), glucagon is predicted as 31% helix and 21% β sheet. From the CD studies of Srere and Brooks (1969), glucagon was found to contain 10–15% helix in dilute aqueous solution (1 mg/ml) which increased to 35% helix at concentrations greater than 10 mg/ml. No conclusion was drawn on the possibility of any β conformation of glucagon in solution by these authors as they did not attempt to calculate the percent β sheet in glucagon from their CD spectra.

β-Sheet Conformation of Glucagon. Recently, Chen et al. (1972) have utilized the CD spectra of proteins with known X-ray structure for determining a new set of refer-

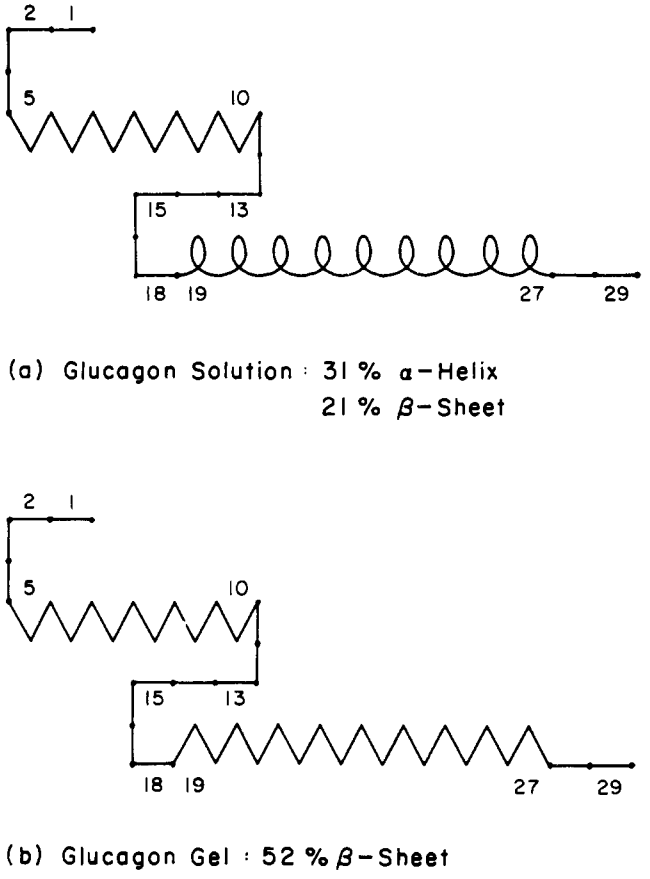

FIGURE 3: Schematic diagram of the secondary structure *predicted* in glucagon. Residues are represented in helical (𝔏), β-sheet (Λ), and coil (⟷) conformational states. Chain reversals denote β-turn tetrapeptides (⊐). Region 19-27 has both helical ($\langle P_\alpha \rangle$ = 1.19) and β-sheet potential ($\langle P_\beta \rangle$ = 1.25) so that the predicted conformation (a) of 31% helix is in agreement with the 35% helix found in concentrated glucagon solutions (10 mg/ml) from CD spectra (Srere and Brooks, 1969). The predicted conformation (b) of 52% β sheet is in agreement with the predominant β structure of glucagon gels found from infrared spectra (Gratzer et al., 1968; Beaven et al., 1969; Epand, 1971).

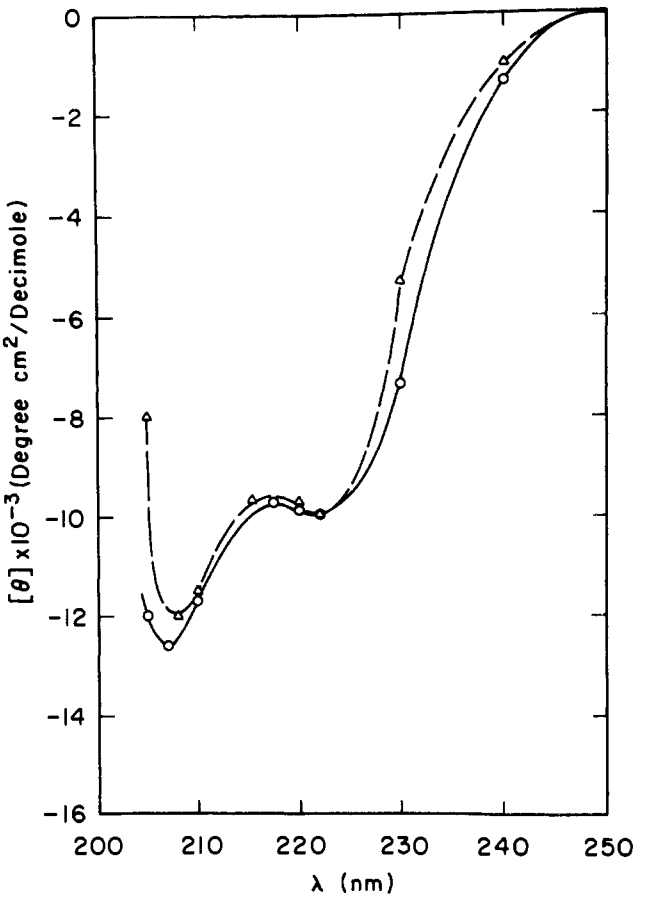

FIGURE 4: Comparison of circular dichroism spectra of glucagon and insulin. The CD of glucagon, 12.6 mg/ml (—), in 10^{-2} *M* Tris-HCl (pH 10.6) taken from Srere and Brooks (1969). The CD of insulin, 1 mg/ml (- - -), at pH 3.0 taken from Ettinger and Timasheff (1971).

Table I: Conformational Prediction for Glucagon: $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, $\langle P_t \rangle$, and p_t Values Computed for Helical, β-Sheet, β-Turn, and Random Coil Regions.

Predicted[a]	$\langle P_\alpha \rangle^c$	$\langle P_\beta \rangle^c$		
1–4 c	0.93	0.87		
5–10 β	0.86	1.08		
11–18 c	0.90	0.91		
19–27 β	1.19	1.25		
28–29 c	0.78	0.93		
Predicted β Turns[b]	$p_t \times 10^{a\,b}$	$\langle P_t \rangle^c$	$\langle P_\alpha \rangle^c$	$\langle P_\beta \rangle^c$
2–5	0.96	1.20	0.83	0.99
10–13	0.88	1.27	0.77	1.01
15–18	0.33	1.21	0.84	0.83

[a] Based on predictive analysis of Figure 1; α = helical, β = β sheet, c = random coil. [b] Based on probability profile of Figure 2. [c] $\langle P_\alpha \rangle$, $\langle P_\beta \rangle$, and $\langle P_t \rangle$ are respectively the average conformational potential for the computed region to be in the helical, β-sheet, and β-turn conformation. $\langle P_\alpha \rangle$ is the sum of the P_α values of the individual residues divided by the total residues in the segment under consideration. $\langle P_\beta \rangle$ and $\langle P_t \rangle$ are derived in a similar manner by averaging the P_β and P_t values, respectively.

ence CD values for the helix, β, and coil conformations, Using $[\theta]_{222} = -10{,}000$, $[\theta]_{210} = -11{,}680$, and $[\theta]_{207} = -12{,}630$ from the CD spectra (Srere and Brooks, 1969) of glucagon at 12.6 mg/ml and eq 1 and Table V of Chen et al. (1972), the values of 33% helix and 20% β sheet in glucagon are calculated. Using the CD curves of poly(Lys) of Greenfield and Fasman (1969), estimates of 24% helix and 16% β sheet in glucagon are obtained. These percentages are in reasonable agreement with the 31% helix and 21% β sheet predicted for glucagon in Figure 3a, and strongly suggest the presence of appreciable β structure in concentrated glucagon solutions. Further supportive evidence of β regions in glucagon can be seen from the similarity of its CD spectra with that of insulin in Figure 4. The molar ellipticities for insulin, $[\theta]_{222} = -9960$, $[\theta]_{208} = -12{,}550$, at pH 3.0 (Ettinger and Timasheff, 1971), and $[\theta]_{222} = -10{,}100$, $[\theta]_{209} = -12{,}300$ at pH 7.0 (Menéndez and Herskovits, 1970) are almost identical with those found for concentrated glucagon solutions (Srere and Brooks, 1969). While Chen et al. (1972) estimated 31% helix and 18% β sheet from their CD studies of insulin, Blundell et al. (1972) found 49% helix and 24% β sheet in their X-ray analysis of insulin. Using the helical wheel method, Schiffer and Edmundson (1970) predicted residues 5–16 and 17–28 as helical in glucagon, and cited their predicted 83% helicity (17% random coil) in agreement with the 75% helical structure of glucagon from preliminary X-ray studies reported by Lipscomb (as quoted in Schiffer and Edmundson, 1970). However, the computed 33% helix and 20% β sheet from the CD spectrum of concentrated glucagon solutions and its resem-

blance to the CD spectrum of insulin which is known to have β structure are in closer agreement with the predicted glucagon conformation (a) in Figure 3. Evidence from infrared spectra shows that concentrated glucagon solutions, which form gels, have a predominant β-sheet conformation in both acidic (Gratzer et al., 1967; Beaven et al., 1969) and basic solution (Epand, 1971). These findings are consistent with our predicted glucagon conformation (b) in Figure 3 which has no helicity but 52% β sheet.

β Turns in Glucagon. From an analysis of the β-turn probability profile of glucagon in Figure 2 as well as comparison of the $\langle P_t \rangle$ values with $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values for the 26 tetrapeptide combinations in this hormone, three β turns are predicted. These regions which show chain reversal in glucagon are shown in Figure 3 as residues 2–5, 10–13, and 15–18. Although nine β turns have been predicted in glucagon by McBride-Warren and Epand (1972), they used the frequency data on only three proteins compiled by Lewis et al. (1971) in computing their probability profile in contrast to the 12 proteins compiled by Chou and Fasman (1974b) and utilized in the present analysis. Hence the three β turns predicted herein for glucagon should have greater reliability. Thus it would appear that this 29 residue polypeptide hormone has the potential to fold into a relatively compact conformation with considerable secondary structure rather than an elongated rod or a structureless random coil. Hence the predicted glucagon conformations (Figure 3) are in agreement with the viscosity studies and hydrogen–tritium experiments of Epand et al. (Epand, 1971; McBride-Warren and Epand, 1972), which indicated that glucagon has a tertiary structure. It is known that β-structure stability has a concentration dependence when it relies on interchain interactions (Davidson and Fasman, 1967; Wooley and Holzworth, 1970). Likewise short helical regions have marginal stability compared to longer helices (Yaron et al., 1971). Thus on increasing the concentration the two conformations suggested in Figure 3 will become more stable, the helices through α–α interchain interaction and at still higher concentration the β structure through interchain association.

Recently a 37-residue peptide was isolated by Tager and Steiner (1973) who proposed it as a possible fragment of proglucagon. The first 29 residues were identical with those of glucagon and the remaining eight residues at the COOH-terminal were found to be Lys-Arg-Asn-Asn-Lys-Asn-Ile-Ala. While these additional residues will neither nucleate helices nor β sheets in the proposed proglucagon (i.e., from the predictive method) they do promote three additional β turns at 28–31 ($p_t = 0.64 \times 10^{-4}$, $\langle P_t \rangle = 1.17$), 30–33 ($p_t = 1.07 \times 10^{-3}$, $\langle P_t \rangle = 1.34$), and 33–36 ($p_t = 0.89 \times 10^{-4}$, $\langle P_t \rangle = 1.24$). Perhaps viscosity and tritium–hydrogen exchange experiments on this possible precursor will verify that it has a more compact folded conformation than does glucagon.

Structure–Function Relationships of Glucagon. Chemical modification and cleavage experiments on glucagon show that the entire molecule is essential for its full biological activity of activating an adenylate cyclase system (Rodbell et al., 1971). This observation is compatible with the proposed structure, as almost all the residues of glucagon are predicted to be in structured regions (helix, β sheet, and β turn) (Figure 3). Spiegel and Bitensky (1969) found that glucagon fragment (1–23) failed to activate the adenylate cyclase system while the fragment (1–27) had diminished biological activity. Of the four glucagon fragments, (1–21), (22–29), (20–29), and (2–29), studied by Rodbell et al. (1971), none were shown to stimulate adenylate cyclase activity, and it was concluded that the region 22–27 is essential for binding of glucagon to its receptor. An examination of the predicted glucagon conformation in Figure 3b shows that residues 22–27 are part of the tail-end β sheet which may bind by forming hydrogen bonds with the receptor site. This could be the hormone conformation necessary for binding to the receptor site which may also be in the β conformation. As neither isolated fragment (20–29) nor (22–29) competes with glucagon at the receptor, Rodbell et al. (1971) proposed that additional interacting residues must exist such as the N-terminal histidine. Since residues 5–10 are also predicted to be β sheet (Figure 3), it is suggested that this region as well as residues 22–27 might be involved in the receptor binding site. The cyanogen bromide cleavage of residues 28–29 at the C-terminal end of glucagon did not eliminate the biological activity of the remaining glucagon fragment (1–27) (Spiegel and Bitensky, 1969). As residues 28–29 were not predicted to be a continuation of the β region 19–27 (the binding site), and were predicted as random, this cleavage would not be expected to disrupt the binding, and biological activity would be retained, as was found. While the removal of the N-terminal histidine of glucagon abolishes its biological activity, fragment (2–29) can still bind with the glucagon receptor (Rodbell et al., 1971). A reasonable explanation may be offered by examining the predicted glucagon model in Figure 3b. It is possible that β turn 2–5 protrudes out of the glucagon molecule like a key in catalyzing adenylate cyclase, and when this key is distorted as a result of cleaving His-1, the full biological activity of glucagon is lost. However, the β regions 5–10 and 19–27 predicted in glucagon remain unaffected in the glucagon fragment (2–29) so that binding to the glucagon receptor is still possible.

$\alpha \rightarrow \beta$ Transition. There is ample evidence that under different concentration conditions, glucagon has various α and β ratios. Thus a transition from α to β appears probable and the residues possibly involved are demonstrated in the predicted forms (Figure 3a and b). As the concentration in vivo will be far from that necessary to elicit the β conformation it is proposed that this conformational state is induced in glucagon upon its association with the receptor site. Thus an induced conformational change from 31% α helix, 21% β sheet (Figure 3a) to 52% β sheet (Figure 3b) is invoked upon binding to the receptor site.

It is interesting that Bornet and Edelhoch (1971) utilized the interaction of glucagon with cationic detergents as a model system for peptide hormone interaction with membranes. From their optical rotatory and fluorescence experiments, it was suggested that glucagon acquires secondary and tertiary structure when combined with detergent. Although no interpretation was made concerning $\alpha \rightarrow \beta$ transitions in glucagon, Bornet and Edelhoch (1971) suggested that "evidently some compositional balance or sequential arrangement of side chains favors the folding of glucagon when its environment is altered." It can now be seen from Figure 3 that the structural sensitivity of glucagon is due to residues 19–27 which has both α-helical ($\langle P_\alpha \rangle = 1.19$) and β-sheet potential ($\langle P_\beta \rangle = 1.25$). Hence, the biological activity of glucagon may reside, not in its conformation in dilute aqueous solution, but in its ability to assume a folded conformation upon interaction with its specific receptors. The $\alpha \rightarrow \beta$ transition for glucagon proposed herein enables the hormone to adopt a more compact structure upon recep-

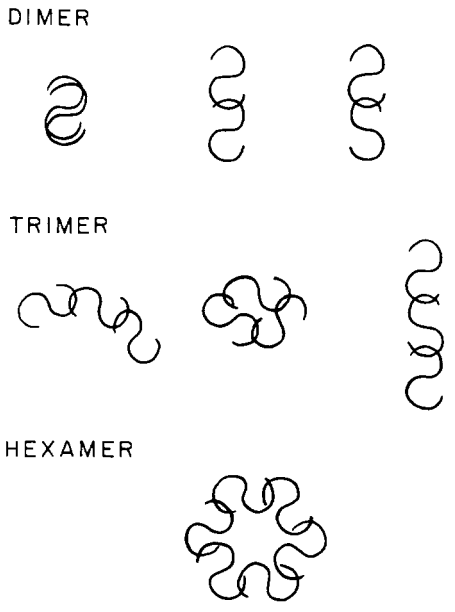

FIGURE 5: Proposed conformational models of glucagon aggregation. The glucagon monomer has roughly a S-type conformational shape with β-sheet regions 5–10 and 19–27 serving as aggregation sites (see Figure 3). These S-shaped glucagon monomers aggregate to form trimers (Blanchard and King, 1966; Gratzer and Beaven, 1969; Gratzer et al., 1972) as well as dimers and hexamers (Swann and Hammes, 1969).

tor binding. This is supported by the fact that the cyanogen bromide peptide of glucagon does not associate to form structures of higher helical content, yet it does form β sheets and is biologically active (Epand, 1972b).

Proposed Modifications of Glucagon. As the conformation of region 19–27 is delicately balanced between the α and β conformations it is predicted that replacement of one or two residues with high β potential in this region with strong α formers would lock the conformation in the α-helical structure. If the above hypothesis for binding to the receptor is correct, namely the necessity of the β structure, then this homolog of glucagon would be without biological activity as a consequence of being unable to bind. A replacement of Val-23 to Glu would raise $\langle P_\alpha \rangle$ from 1.19 to 1.23 while $\langle P_\beta \rangle$ would be lowered from 1.25 to 1.09 for the segment 19–27, so that the helical conformation would predominate. A double substitution of residues 22 and 23 from Phe-Val to Glu-Glu results in $\langle P_\alpha \rangle = 1.28$ and $\langle P_\beta \rangle = 0.98$ for region 19–27. This double modification not only further strengthens the helical potential of this region, but prevents formation of the β structure (since $\langle P_\beta \rangle < 1$). Another interesting substitution would be to interchange Phe-6 to Glu in the other predicted β region 5–10, resulting in $\langle P_\alpha \rangle$ changing from 0.86 → 0.93 and $\langle P_\beta \rangle$ changing from 1.08 → 0.91, causing this β-sheet region to assume a random coil conformation. If the β structure at residues 5–10 is involved in the receptor-binding site, such a modification would be reflected in the loss of biological activity of glucagon. Experimental studies of these modified glucagon homologs should be extremely rewarding.

The aggregation of glucagon still persisted in carboxyl group modified form as demonstrated in the concentration dependence of CD and ultracentrifuge studies (Epand and Epand, 1972). It was suggested that although the carboxyl groups of glucagon (Asp-9, -15, and -21) are important for membrane binding, this is not the sole requirement for binding since competition experiments between poly(Glu) or between poly(Asp) and glucagon showed no inhibition of glucagon activation of adenylate cyclase (Epand and Epand, 1972). Since Asp-9 and Asp-21 are within the predicted β regions 5–10 and 19–27, blockage of these carboxyl groups apparently did not hinder the ability of β-sheet aggregation, although the modified glucagon did lose biological activity. On the other hand, the inability of poly(Glu) and poly(Asp) to inhibit glucagon-receptor binding strongly suggests that the β conformation is equally as important as the carboxyl groups in glucagon for this essential binding. Since Glu [$(P_\alpha)_{Glu} = 1.53$, $(P_\beta)_{Glu} = 0.26$] and Asp [$(P_\alpha)_{Asp} = 0.98$, $(P_\beta)_{Asp} = 0.80$] have stronger helical than β potential (Chou and Fasman, 1974b), it is difficult for poly(Glu) and poly(Asp) to assume the β conformation.

Glucagon Aggregation. Glucagon is known to undergo aggregation and this phenomenon has been studied from several points of view. Gratzer and Beaven (1969) found that Tyr-13 undergoes a pK_a shift from 9.9 to 10.1 upon glucagon association while Tyr-10 remains unaffected with a pK_a of 10.7 for both disaggregated and associated forms.

Nuclear magnetic resonance studies of 20-mg/ml glucagon solutions by Patel (1970) showed the hormone to be in the aggregated state rather than the helical state at the C-terminal end (although it is not apparent why one cannot have an aggregated helical state), and it was proposed that glucagon residues 14–29 aggregate as a tail-to-tail dimer. Based on sedimentation equilibriums and gel filtration studies, Swann and Hammes (1969) postulated formation of glucagon dimers and that the dimerized helices aggregate further to former hexamers. Rod-like trimers of glucagon helices have also been proposed (Blanchard and King, 1966; Gratzer and Beaven, 1969; Gratzer et al., 1972). An examination of the proposed glucagon conformation in Figure 3b shows that the molecule has basically an S-shape with β-sheet regions 5–10 and 19–27 forming the head and tail, respectively. It is proposed that these two β-sheet regions, rather than helices, are involved in head-to-tail as well as tail-to-tail glucagon aggregation. For sake of clarity, the β turn 2–5 has been omitted in sketching the S-shaped glucagon monomer unit to form the dimer, trimer, and hexamer models of glucagon aggregation in Figure 5. Intermolecular β-sheet hydrogen bonding is suggested at the contact sites of the S-shaped glucagon monomers in forming aggregates. Since aggregation of insulin dimers and hexamers are known to involve intermolecular β sheets from X-ray studies (Blundell et al., 1972), a similar mechanism for aggregation of glucagon β sheets appears plausible from the prediction model.

Addendum

After the submission of this manuscript, a paper [Panijpan and Gratzer, 1974] presented further evidence against the globular nature of monomeric glucagon.

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