Immunochemical Studies on Human Calcitonin M Leading to Information on the Shape of the Molecule

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1. Two antisera were obtained from a single rabbit. Both are highly specific for human calcitonin M but react with different parts of the amino acid sequence. 2. The different sequences that react with the antibodies of the two antisera were located. The first antiserum reacts at two sites in the molecule, one in the sequence residues 11–18, probably with residue 17 as the immunodominant group, and another on either side of the 28–29 peptide bond. The second antiserum, harvested 9 months later, reacts principally at one site bridging the 28–29 peptide bond. 3. A consideration of the properties of the hormone's binding sites and of data relating biological activity to structure enables some conclusions to be drawn with regard to the shape of the molecule. It appears that the peptide chain is folded to bring *N*- and *C*-termini closer together and that there is non-covalent interaction between regions in the chain near both termini. One of these is located near residue 8.

Antigen–antibody binding is due to the fitting of complementary shapes on the two molecules with consequent van der Waals and ionic interactions. This makes binding energies very sensitive to conformational changes in the antigen (Sela *et al.*, 1967; Goodman, 1969). In some instances antibodies to a native protein may not react with the denatured protein (Sela *et al.*, 1967; Brown, 1963) and, similarly, antibodies to a denatured protein may be obtained that do not react with the native protein (Sela *et al.*, 1967; Brown, 1962). Hence changes in binding energy may be used as a tool to investigate the conformation of the antigen. However, care must be taken in interpretation since the loss of an amino acid residue from one end, say, of an antigenic site may decrease binding energy because one of the primary sites of van der Waals interaction has gone rather than through a conformational change. Equally, a change in binding energy on chemical modification of a side chain could be due to its being involved either directly in binding or secondarily in a conformational role. Other facts are required to choose between the alternatives.

Several such studies have been made. Atassi & Saplin (1968) showed that the conformation of antigenic sites on the three cyanogen bromide fragments of myoglobin was the same as in the native protein, since the sum of their immunoreactivities equalled that of the whole protein. More extensive fragmentation by chymotrypsin (Crompton & Wilkinson, 1965) resulted in diminished reaction with antibody as more of the three-dimensional structure was destroyed. Chymotryptic cleavage within reactive sites themselves could, of course, account for a part of

the lack of reactivity. This dependence on conformation in binding to antibodies has also been noted in a small peptide. Spragg *et al.* (1968) proved that the natural conformation of the antigenic site of bradykinin was achieved only if the peptide contained the full nine residues.

Several other workers (Dietrich & Rittel, 1970; Lequin *et al.*, 1969; Tashjian *et al.*, 1970; Potts *et al.*, 1970) have raised antisera to pig or human calcitonin and have studied in varying detail the regions within the hormone's sequence to which antibodies bind. Most find central or *C*-terminal antigenic sites; the antiserum reported by Potts *et al.* (1970) reacts with the *N*-terminal sequence residues 1–9. In addition, some of the studies demonstrate that the *N*-terminal region affects the conformation of the antigenic sites.

The calcitonins show great differences in amino acid sequence between the species. In the five sequences so far determined (human, cow, pig, sheep and salmon) only nine of the 32 residues are conserved: those at positions 1, 3, 4, 5, 6, 7, 9, 28 and 32. Surprisingly, however, the complete sequence is required for biological activity (Sieber *et al.*, 1970).

This present study was carried out to find the antigenic sites in human calcitonin M (the monomeric form of the hormone) that react with two antisera. It was also hoped that this might throw light on the shape of human calcitonin M.

Experimental

Production of antisera

For the initial immunization a 10mg portion of a crude preparation of human thyroid medullary

carcinoma (Byfield *et al.*, 1968) containing approx. 10% of the hormone was dissolved in 1 ml of water and adsorbed on to 50mg of finely divided carbon [Statex E12; Columbian International (Great Britain) Ltd., Four Ashes, Wolverhampton, Staffs., U.K.]. The suspension was then emulsified with 2ml of complete Freund's adjuvant and injected into each inguinal and popliteal lymph node (0.5 ml/node) and the spleen (1 ml) of a 1500g New Zealand white rabbit.

Booster injections of 5mg portions of the crude hormone similarly prepared with 0.5ml of water, 25mg of carbon and 1ml of complete Freund's adjuvant were injected into the thigh muscles at approx. 2-month intervals during 8 months. At the end of this time the first antiserum (antiserum I) was obtained by bleeding from an ear vein. Boosting was then continued at approx. 2-month intervals with 5mg quantities of pure synthetic human calcitonin M prepared for injection exactly as for the crude hormone and injected into the thigh muscles. After a further 9 months the second antiserum (antiserum II) was obtained by bleeding from an ear vein.

Both antisera give identical displacement curves with human calcitonin M when used at similar dilutions but react differently with peptide fragments of the hormone (Fig. 1). Neither antiserum shows detectable reaction with pig, salmon, chicken or rat calcitonins, bovine parathyroid hormone, oxytocin, vasopressin, bradykinin or synthetic β -1-24-adrenocorticotrophin when these peptides are tested at 1000-fold higher molar concentrations than those of the reactive fragments of human calcitonin M.

Preparation of human calcitonin M

The pure hormone used as a standard in the radioimmunoassay and for the preparation of the radioactively labelled tracer, enzymic fragments and derivatives was isolated from a further human thyroid medullary carcinoma. The method is described in detail by Byfield *et al.* (1970). The hormone was judged pure from its amino acid composition and by demonstrating its homogeneity in the following nine t.l.c. systems. On alumina [Alox D-O, Camag A.G., Muttentz, Switzerland, plus 8.4% (w/w) CaSO_4]:1, butan-2-ol-3% (w/v) NH_3 (7:3, v/v), R_F 0.54; 2, butanol-pyridine-acetic acid-water (21:12:2:15, by vol.), R_F 0.56; 3, butanol-pyridine-acetic acid-water (17:12:6:15, by vol.), R_F 0.59; 4, butanol-pyridine-water (1:1:1, by vol.), R_F 0.55; 5, butanol-acetic acid-water (50:5:14, by vol.), R_F 0.54. On cellulose (Avicel microcrystalline cellulose; Avicel/FMC Corp., Newark, Delaware, U.S.A.): 6, butanol-pyridine-acetic acid-water (21:12:2:15, by vol.), R_F 0.59; 7, butanol-pyridine-water (1:1:1, by vol.), R_F 0.67; 8, ethyl acetate-pyridine-acetic acid-water (62:21:6:11, by vol.),

Fig. 1. Displacement curves of human calcitonin M (11-28)-octadecapeptide in reaction with antiserum I (○) and antiserum II (△) compared with that for the whole hormone (●)

R_F 0.73. On silica (silica gel G, Merck, Darmstadt, German Federal Republic.); 9, butan-2-ol-3% (w/v) NH_3 (7:3, v/v), R_F 0.38.

Isolation of immunoreactive fragments of human calcitonin M

Preliminary studies had shown that cleavage by trypsin, pepsin and chymotrypsin gave immunoreactive fragments of the hormone.

Enzyme digestions were carried out at 37°C with enzyme/substrate ratios of 1:50 (w/w) and substrate concentrations of 1mg/ml. Reaction was stopped by cooling to -78°C. Solvent and buffering agents were then removed by freeze-drying. Suitable controls showed no interference by residual enzymes in subsequent radioimmunoassays.

Pepsin (batch 33030; Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.) was used in 1M-formic acid. Trypsin and chymotrypsin were used in 0.5M-ammonium carbonate. Two preparations of trypsin were used. For specific cleavage at the lysine-18 residue a sample of diphenylcarbamoyl chloride-treated trypsin (batch 108B-8070; Sigma Chemical Co., St. Louis, Mo., U.S.A.) was employed. An untreated preparation of trypsin (batch 42788; Koch-Light Laboratories Ltd.) contained a chymotryptic-like contaminant, to which the tyrosine-12 residue in the hormone is exceptionally sensitive. This proved advantageous, however, as chymotryptic digestions were too rapid to be easily controlled. When the lysine-18 side chain of human calcitonin M had been blocked by acrylonitrile the preparation could be used to give specific cleavage at tyrosine-12.

Peptide H-9-32- NH_2 was isolated from a 60min peptic digest of the hormone by gel filtration on a small-pore polyacrylamide gel (Bio-Gel P-4; Bio-Rad

Laboratories, Richmond, Calif., U.S.A.). Peptide H-19-32-NH₂ was obtained from a digest made with the trypsin preparation containing chymotryptic activity. Extended digestion (2.5h) with such preparations yields a mixture of peptides of sequences residues 1-12, 13-18 and 19-32. Countercurrent distribution in the system butan-1-ol-pyridine-water (10:1:10, by vol.; nine transfers) followed by gel filtration on Bio-Gel P-4 then gave the pure fragment H-19-32-NH₂. Peptide H-13-32-NH₂ was isolated after blocking the lysine-18 side chain amino group with acrylonitrile and then obtaining the chymotryptic cleavage at tyrosine-12 with the untreated trypsin preparations mentioned above. The fragments of sequences residues 1-12 and 13-32 were separated by gel filtration on Bio-Gel P-4. Cyanoethylation was shown not to affect binding to antibodies (Figs. 3 and 4), which justifies ignoring its effect in this sequence.

The isolated peptides were identified via their amino acid compositions and comparison with the sequence of the hormone (Neher *et al.*, 1968).

Peptide H-11-18-OH was obtained from the synthetic peptide H-11-28-OH by digestion for 30min with diphenylcarbamoyl chloride-treated trypsin. It was not separated from the peptide H-19-28-OH.

Preparation of derivatives

Oxidation with performic acid was carried out essentially as recommended by Hirs (1956). The reagent was made by mixing formic acid and hydrogen peroxide (30g/100ml) in the ratio 19:1 (v/v) 2h before use. It was then allowed to react with the peptide (2ml/mg) for 4h at 4°C and removed *in vacuo* over P₂O₅ and NaOH.

Cyanoethylation was carried out as described by Fletcher (1966). Redistilled acrylonitrile (1ml) was added to 5mg of peptide dissolved in 4ml of 0.1M-triethylamine. After overnight reaction at room temperature the solvent and reagent were removed *in vacuo* over P₂O₅ and NaOH. Amino acid analysis showed that the terminal and ϵ -amino groups were always totally cyanoethylated whereas the imidazole side chain of histidine-20 was substituted to variable extents.

Radioimmunoassay

The reaction of peptide fragments of human calcitonin M and its derivatives with antibodies raised to the whole hormone was assessed by the radioimmunoassay method described by Clark *et al.* (1969).

The assay is a competitive system in which test peptides and ¹²⁵I-labelled hormone compete for antibody binding sites. By serial dilution various con-

centrations of test peptides were obtained and examined for their competition with a constant amount of the radioactive tracer. The percentage of radioactivity bound to antibodies at each of several concentrations of test peptide was plotted to give a characteristic displacement curve for that peptide. In duplicate determinations the values of tracer bound to antibody usually agreed to within 3%. Antisera I and II were used in the test mixtures at final dilutions of 1:2700 and 1:3000 respectively.

Amino acid analysis

Amounts between 0.05 and 0.1 mg of peptides were hydrolysed in 0.2ml of 6M-HCl under N₂ in sealed tubes at 110°C for 24h. Analysis was carried out on a sub-micro amino acid analyser (Technicon Instruments Co. Ltd., Chertsey, Surrey, U.K.), with a resin column (3mm×1500mm) and the buffer system described by Thomas (1967).

Synthetic fragments of human calcitonin M

A number of synthetic fragments of the hormone were kindly supplied by Dr. W. Rittel of Ciba/Geigy Ltd., Basle, Switzerland. They represented the sequences residues 1-10, 11-16, 11-28, 11-32, 17-28, 21-28 and 29-32 within the 32-residue peptide amide. In addition he gave a sample of synthetic [8-methionine sulfoxide]-human calcitonin M.

Results

The percentage of ¹²⁵I-labelled calcitonin M bound to antibody in the presence of a test peptide was taken as a measure of the peptide's reaction with human calcitonin antibodies. The displacement curve was compared with the curve obtained with the native hormone and, if identical, the test peptide was assumed to possess all the antigenic sites of the hormone in their native state.

The binding of an antigen to its antibody is due to the fitting of complementary shapes on antigen and antibody. Any change in the surface shape of an antigen by chemical modification of a group, or by a change in the conformation of the molecule in that region, will be reflected in a change in the binding energy of the antigen. This in turn will be reflected in a change in the displacement curve given by the test peptide. The result is a difference in the molar concentration of peptide required to achieve a given degree of displacement of radioactive tracer from antibody compared with that required with the native antigen (see, e.g., Fig. 4).

In an extreme case the chemical modification or conformational change in an antigenic site may be so severe that the binding energy is decreased below detectable amounts. In this case the test peptide will

Fig. 2. Displacement curves of a selection of fragments of human calcitonin M in reaction with antiserum I compared with that for the whole hormone

□, Peptide H-1-10-OH; ▲, peptide H-11-16-OH; ○, peptides H-11-18-OH and H-11-28-OH; △, cyanoethylated peptide H-11-28-OH; ■, peptide H-11-32-NH₂; ▽, peptide H-17-28-OH; ▼, peptide H-21-28-OH; ×, peptide H-29-32-NH₂; ●, whole hormone.

apparently not compete with radioactive tracer for binding sites on antibodies specific for the antigenic site and no displacement of tracer will be seen. A similar result will, of course, also be obtained with a test peptide wholly lacking the sequence of the antigenic site so that the situations of extreme modification in an antigenic site and its total absence from a peptide fragment cannot be distinguished in this single test and further information is required to determine the actual case.

When an antiserum reacts with two or more sites in an antigen a further effect may be seen in its reaction with peptide fragments of the antigen. The observed displacement curve of the original antigen in reaction with this antiserum is the sum of several curves representing the reaction of the different antigenic sites with their specific antibodies. A peptide fragment of the original antigen lacking one of the antigenic sites, or with one so severely modified that its binding energy is undetectable, will be unable to displace radioactive tracer from antibodies specific for the lost (or modified) site. It will, however, still react with all other antibodies. Nevertheless, a portion of the radioactive tracer normally displaced remains bound to antibody and the maximum displacement of tracer achieved with the whole antigen cannot be reached by the test peptide. Its displacement curve therefore reaches a plateau at a higher value of percentage radioactive tracer bound to antibody compared with that given by the whole antigen, e.g. see the displacement curve of peptide H-11-28-OH in Fig. 2.

Any changes in the antigen that have no effect on

its binding to antibodies may be assumed not to involve or influence the antigenic sites.

Antiserum I

The displacement curve of several peptides in reaction with antiserum I are shown in Figs. 2 and 3. A peptide comprising the sequence residues 11-32 of human calcitonin M achieves maximum displacement of ¹²⁵I-labelled calcitonin from antibody and gives a reaction curve apparently identical with that of the whole hormone (Fig. 2). It therefore contains all the antibody-binding sites of the hormone in their native state.

Peptide H-11-28-OH reaches only half-maximal displacement of radioactive tracer and consequently lacks an antigenic site(s) or part of a site that must be contained in the C-terminal region of the hormone. Peptide H-29-32-NH₂, however, does not react with antiserum I at similar concentrations, and thus the missing antigenic site must involve residues on both sides of the 28-29 peptide bond, unless the sequence residues 29-32 are essential for the correct conformation of a site within the sequence residues 11-28.

The second antigenic site is present in the sequence residues 11-28. It is probably wholly within the sequence residues 11-18, as a peptide of this sequence gives a reaction curve identical with that of the sequence residues 11-28 (Fig. 2). However, as peptide H-11-16-OH gives no displacement of tracer the binding site is at the C-terminus of the sequence residues 11-18. The ε-amino group of lysine-18 is not involved, since its blocking with acrylonitrile in the

sequence residues 11–28 (Fig. 2) and whole hormone (Fig. 3) does not affect reaction with antiserum I. Residue 17 (asparagine) may therefore be the immunodominant group. Alternatively residues 17 and 18 may be essential for the correct conformation of the site.

The displacement curves of oxidized derivatives of the full sequence are shown in Fig. 3. The same curve was obtained for both the [1,7-dicysteic acid, 8-methionine sulphone]-human calcitonin M produced by performic acid oxidation and the synthetic [8-methionine sulfoxide]-human calcitonin M. The

oxidized derivatives have a raised binding energy, since they give the same percentage of tracer bound to antibody at lower peptide concentrations than the native hormone. Cleavage by oxidation at the 1–7 disulphide bond will produce ring distortion by mutual repulsion of the resultant sulphonic acid groups. It appears, however, not to alter the effect of oxidation at methionine-8 alone. Two conclusions may be drawn from this result. First, the disulphide bond itself and the residues of the ring system do not bind to the antibodies, nor do they interact with antigenic sites on the hormone. Secondly, the region of the methionine residue (position 8) is important either in binding to the antibodies or in interactions that affect the shape of antigenic sites on the hormone. The latter possibility is favoured, since it is shown by the results of Fig. 2 that all antibody-binding sites are in the sequence residues 11–32.

Antiserum II

Studies with antiserum II are presented in Fig. 4. The peptides representing the sequences residues 9–32, 13–32 and 19–32 of the hormone gave identical displacement curves, with maximum displacement of ^{125}I -labelled human calcitonin M from antibodies in antiserum II. Clearly, therefore, the common sequence residues 19–32 contains all the antigenic site(s). The binding energy of the site is less than that of the whole hormone, since the reaction curve is displaced to higher peptide concentrations. A peptide representing the sequence residues 11–28 of the hormone gave very little reaction with antiserum II, indicating that the reactive site extends beyond 28 or

Fig. 3. Displacement curves of cyanoethylated human calcitonin M (Δ), [8-methionine sulfoxide]-human calcitonin M ($\blacksquare$), and performic acid-oxidized human calcitonin M ($\square$), in reaction with antiserum I compared with that of the native hormone ($\bullet$)

Fig. 4. Displacement curves of fragments of human calcitonin M and whole hormone and cyanoethylated human calcitonin M in reaction with antiserum II

□, Peptide H-9-32-NH₂; ○, peptide H-11-28-OH; ▽, peptide H-13-32-NH₂; ▲, peptide H-19-32-NH₂; ●, whole hormone; Δ, cyanoethylated human calcitonin M.

requires the sequence residues 29–32 for its correct conformation. The free peptide H-29–32-NH₂ does not react with this antiserum even at 1000000-fold higher concentrations than those shown for reactive peptides in Fig. 4.

As was found with antiserum I, cyanoethylation of the hormone at the *N*-terminal amino group and the side chains of lysine-18 and histidine-20 did not affect binding to antibody.

Discussion and Conclusions

The results are consistent with antiserum I containing two distinct populations of antibodies. One reacts with the *C*-terminal section of the hormone straddling residues 28 and 29. The second reacts with a central portion of the hormone either side of residues 16 and 17, probably extending no further than residue 18. The ϵ -amino group of lysine-18 does not interact with antibody, and residue 17 (asparagine) may be the immunodominant group. Antiserum II, in contrast, contains one major population of antibodies reacting with the *C*-terminal region of the hormone on either side of residues 28 and 29. It is not clear, however, if the *C*-terminal antigenic sites found for each antiserum are identical. For antiserum I the peptide H-11–32-NH₂ gave a reaction curve identical with that of peptide H-1–32-NH₂, whereas with antiserum II peptide H-9–32-NH₂ (similar to peptide H-11–32-NH₂) gave a reaction curve different from that of the full sequence H-1–32-NH₂. But the situation is confused by there being two reacting sites in antiserum I.

The population of human calcitonin antibodies in the rabbit changed markedly over the 9 months between the harvesting of antisera I and II. A similar change with time in one rabbit has also been observed in antisera to tobacco-mosaic-virus protein (Benjamin *et al.*, 1968). In that case it was shown that there was a common sequence in all the antigenic sites on the protein, with each antiserum requiring different additional residues. The sequences became shorter with time. In the present investigation two binding sites were observed initially, one of which decreased in importance with time.

There is no ready explanation for this change in antibody population. There are at least two apparent explanations, neither of which withstand detailed examination. First, both antisera I and II contain only immunoglobulin G antibodies (P. A. Kendall, personal communication), so that the effect is not likely to be the changeover from immunoglobulin M to immunoglobulin G antibodies (with a different specificity), as this would have occurred well before antiserum I was harvested (8 months after initial immunization). Secondly, the results could be explained if the synthetic human calcitonin M used for later stages of boosting was different from the

natural hormone in being immunogenic only at the *C*-terminus. Dietrich & Rittel (1970) raised antibodies to synthetic human calcitonin M in several rabbits and each antiserum reacted with the *C*-terminus of the hormone only. However, natural and synthetic human calcitonin M molecules give identical curves when assayed with either antiserum used in this study. Therefore with antiserum I, which was raised to the 'natural' hormone alone, the synthetic hormone must also have reacted with that antibody population directed to the central binding site. Moreover the peptides H-11–18-OH, H-11–16-OH and H-11–28-OH used to study this site were also synthetic, derived from the synthetic route to human calcitonin M (Sieber *et al.*, 1968). Thus a difference in the chemical structure of the natural and synthetic hormone used to challenge the rabbit cannot explain the change in antibody population.

In the sequences of the five calcitonins so far known there are only nine conserved residues of the 32, namely 1, 3, 4, 5, 6, 7, 9, 28 and 32. The antigenic sites of human calcitonin M detected in this study are all in the region of the hormone that (except for glycine-28) is non-conserved. Antigenic sites are most often found in non-conserved regions of peptides and proteins (Schwyzer, 1968).

A consideration of the results in Figs. 2, 3 and 4 suggests some features of the structure of the calcitonin molecule. Taken with other facts on the relationship between structure and biological activity in the calcitonins, a tentative model for the shape of the molecule may be described.

Results on the relationship of biological activity to structure in the calcitonin molecule indicate a close relationship between *N*- and *C*-termini. As seven of the nine conserved residues lie at the *N*-terminus they are no doubt intimately concerned with biological activity. This is confirmed by the observations that cleavage of the 1–7 disulphide bond in pig calcitonin (Brewer, 1969) and oxidation of methionine-8 in human calcitonin (Riniker *et al.*, 1968) destroy biological activity. However, the *C*-terminal proline amide residue is also essential for full biological activity (Sieber *et al.*, 1970), which is best explained if *N*- and *C*-termini are close in space. It is noteworthy, however, that many substitutions in the intervening sequences are tolerated and some of these increase the biological activity in assay animals.

It was shown in Fig. 4 that the binding energy of the antigenic site to antiserum II was decreased when the *N*-terminal region was absent from test peptides. Loss of binding energy could be due to a change in conformation or loss of a residue from the antigenic site. In the former case the *N*-terminal region would interact with another part of the molecule in such a way as to stabilize a particular conformation in the *C*-terminal antibody-binding site. In the latter case the *N*-terminus would supply a necessary residue to

the site. Either reason suggests an interaction between the C-terminal antigenic area and the N-terminal region supporting the conclusion deduced from biological data.

One point of interaction between the two ends of the chain is near residue 8. This is apparent from the results in Fig. 3, where it is seen that oxidation of the sulphur-containing residues 1, 7 and 8 increased the total binding energy of the hormone to the antibodies of antiserum I. It is not possible that the oxidized derivatives react solely with an alternative set of antibodies in the antiserum, since both the native and oxidized hormone displace more than 85% of the same radioactively labelled tracer from antibody at about 3 pM concentration. Therefore, the tracer, native hormone and oxidized derivatives are all reacting predominantly with the same antibodies. It is possible that there is an additional small concentration of antibodies that react with oxidized human calcitonin but not with the native hormone. Alternatively the oxidized derivatives may merely bind more strongly than the native hormone to the same set of antibodies. Either phenomenon would be explained by a conformational change brought about by oxidation of the sulphur-containing residues. The same effect was seen whether the methionine-8 residue alone or it plus half-cystine-1 and half-cystine-7 were modified, implying that the effect is only due to oxidation at the methionine residue (and that scission of the disulphide bond has no effect on binding energy). The region around residue 8 must therefore be involved with the conformation of the antigenic sites of the hormone that react with antiserum I. There are two such sites, but the C-terminal one is favoured in view of the evidence presented above for interactions between N- and C-termini. The nature and position of the involvement of the region of residue 8 with the C-terminal sequence cannot be determined from these results. It may be indirect, being mediated through an intermediate sequence.

The residues of the disulphide ring system are probably not involved in interaction elsewhere. Scission of the disulphide bond by performic acid results in two mutually repelling sulphonate acid groups, which would distort the residues of the ring and any others with which they may be interacting. Support for this is found in circular-dichroism studies on bovine calcitonin by Brewer (1970). He found that the disulphide ring is relatively flexible, which it would not be if it were interacting elsewhere.

Our proposed model is shown schematically in Fig. 5. The molecule is folded to bring N- and C-termini closer together. The disulphide ring system is free of interactions with the rest of the molecule. The region around residue 8, however, interacts with a C-terminal sequence to influence the conformation of the C-terminal antigenic sites.

The method used to derive this model does not

Fig. 5. Tentative representation of the shape of calcitonin

The amino acid sequence of human calcitonin M is: H-Cys-Gly-*Asn-Leu-Ser-Thr*-Cys-Met-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-*Pro-NH₂*, where only the italicized residues are conserved in the hormone from man, pig, cow, sheep and salmon.

approach the detail possible with X-ray crystallography, but, equally, the crystal structure of a molecule may differ from that in solution. Therefore, a method such as this, which can give information on the solution state, is of value.

There are two features of the binding of a hormone with its antibody or receptor site. They are the recognition of the hormone by the binding molecule and the excellence of fit of the relevant surfaces on hormone and binding molecule. Although the first point is related directly to conformation in solution, the second point may be less directly related, since the action of binding may induce conformational changes that assist fitting of the binding surfaces. However, since the same general shape was deduced from consideration of binding at antigenic and biologically active sites, it seems likely that our model represents the shape in solution that is relevant to physiological reactions.

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