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THE cDNA-DERIVED AMINO ACID SEQUENCE OF CHICK HEAT SHOCK PROTEIN M_r 90,000 (HSP 90) REVEALS A "DNA LIKE" STRUCTURE: POTENTIAL SITE OF INTERACTION WITH STEROID RECEPTORS

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SUMMARY: We report cDNA sequence, the complete derived aa sequence, and a predicted secondary structure of the chick hsp 90, a protein which has been found to form complexes with steroid hormone receptors. The modelling of the most negatively charged "region A" indicates that the α-helices of this portion of hsp 90 mimick DNA configuration. We propose that this region can, in absence of hormone, interact with and cap the positively charged DNA-binding domain of steroid receptors.

The heat shock protein of 90kDa (hsp 90) is a cytosol protein of unknown function abundant at physiological temperature. Changes of its expression during differentiation as well as post-translational modifications have been described (1,2,3,4). Hsp 90 interacts with and may modulate the function of other proteins such as actin (5), heme-regulated eIF2α kinase (6), tyrosine kinase oncogene products (7) and steroid hormone receptors (8-11).

Steroid receptors are transcriptional regulators which display a common structural organization (12). Two forms have been described for each receptor, with sedimentation coefficients of ~ 4S and ~ 8S. The larger 8S "non active" form, observed in absence of hormone does not bind to DNA, contrary to the smaller "transformed" one (4S). The 8S form includes the hormone binding subunit and hsp 90 ; it can be dissociated in high salt medium, suggesting ionic interaction between the two subunits. The released 4S receptor then can bind DNA (13). It has been suggested that this separation also occurs physiologically in the cell, upon steroid hormone binding (14).

We report here the cloning of chick hsp 90 cDNA and the derived aa sequence. The predicted secondary structure of the protein suggests a model

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where one of the possible interaction of hsp 90 with steroid receptors occurs between the most negatively charged "region A" of the heat shock protein, which mimicks the DNA configuration, and the positively charged DNA binding region of the receptors.

MATERIALS AND METHODS

Construction and screening of cDNA libraries, cDNA characterization and sequencing. A pUC 8/9 library (15) and a λgt11 library (16) from chick oviduct were screened with hsp 90 cDNA inserts of the following recombinant plasmids: p801, containing the human hsp 90 cDNA (17); chick p9.11, previously described (9); and p8.14, characterized in this work. Restriction maps were determined by single and double enzyme digestion. DNA fragments analysed on 1% agarose gels in TBE buffer, were transferred to nitrocellulose and hybridized to radioactively labelled cDNA inserts under stringent conditions (18). Overlapping cDNA inserts were subcloned into M13mp18 and M13mp19 vectors, and sequenced on both strands (19).

Predicted secondary structure.Computer stereodrawing of a modelled protein segment. The secondary structure was predicted using a procedure which combines three different methods (20), and has overall accuracy of 65.5% for the prediction of α-helix, β-strand and coil structures.

The modelling was performed on a PS 390 Evans and Sutherland computer with the MANOSK program (21).

RESULTS

Isolation of chick hsp 90 cDNA. Only a 5' portion of the chick hsp 90 cDNA (p9.11) was cloned from the pUC 8/9 library, since the EcoR1 cloning site was an internal cDNA site (9). The hsp 90 cDNA human clone p801 (17) recognized a chick mRNA of the same size as that recognized by p9.11.

With the p801 insert, twenty-four recombinant clones were isolated from pUC 8/9 library. Three clones were analysed in detail; they hybridized with a ~ 3kb mRNA, as previously described for p9.11. The restriction maps of the three clones were identical, and one clone (p8.14) was selected for sequencing.

To make sure that the EcoRI site was an unique internal site of the hsp 90 cDNA, we screened a λgt11 library from estrogen-stimulated chick oviducts with p9.11 and p8.14 cDNA inserts. From the 200 positive clones, two different clones overlapping the EcoRI site were selected. Sequence analysis on both strands confirmed that the EcoRI site was unique in the chick hsp 90 cDNA.

Nucleotide sequence of the chick hsp 90 cDNA and aa sequence. The nucleotide sequence and the derived aa sequence of hsp 90 are shown in Figure 1. A single open reading frame of 2184 nucleotides starts at the initiator codon ATG and extends to the in-frame termination codon TAA. The predicted molecular size of the chicken hsp 90 is 84,024 daltons; the calculated pI of 5.2, is in agreement with the experimental determinations (11). Two potential sites of phosphorylation by cAMP-dependent kinase at aa 210 and 456, two consensus

CCCTTCCCCCGCTGCCAAG

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ATG CCG GAA GCT GTG CAA ACA CAG GAC CAA CCA ATG GAG GAG GAA GTG GAG ACC TTT GCC TTC CAG GCT GAG ATT GCT CAG TTG ATG TCT
Met Pro Glu Ala Val Gln Thr Gln Asp Gln Pro Met Glu Glu Glu Val Glu Thr Phe Ala Phe Gln Ala Glu Ile Ala Gln Leu Met Ser  30
 C  C  C  H  H  C  C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H

CTG ATT ATC AAC ACT TTT TAC TCC AAT AAG GAA ATC TTC TTG AGG GAA CTG ATC TCC AAT TCA TCT GAT GCT CTG GAC AAG ATC AGA TAT
Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu Leu Ile Ser Asn Ser Ser Asp Ala Leu Asp Lys Ile Arg Tyr  60
 H  E  E  C  H  C  C  C  C  C  H  H  H  H  H  H  H  H  H  C  C  C  H  H  H  H  H  H  H  H  H

GAG AGT TTG ACT GAC CCG AGC AAG CTG GAT TCT GGA AAA GAC CTG AAA ATT AAC CTG ATT CCA AAC AAG CAC GAT CGC ACT CTG ACC ATT
Glu Ser Leu Thr Asp Pro Ser Lys Leu Asp Ser Gly Lys Asp Leu Lys Ile Asn Leu Ile Pro Asn Lys His Asp Arg Thr Leu Thr Ile  90
 H  C  C  C  C  C  C  C  C  C  C  H  H  C  H  E  H  E  C  C  C  C  C  C  C  E  E  E  E

GTG GAT ACC GGC ATA GGG ATG ACC AAA GCT GAC CTT GTC AAC AAT CTT GGT ACT ATT GCC AAG TCT GGT ACC AAG GCT TTC ATG GAA GCA
Val Asp Thr Gly Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr Ile Ala Lys Ser Gly Thr Lys Ala Phe Met Glu Ala 120
 E  E  H  C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H  H  H  C  C  C  H  H  H  H  H  H

CTG CAG GCA GGG GCT GAT ATT TCC ATG ATT GGT CAG TTT GGT GTT GGT TTC TAC TCT GCT TAC CTT GTT GCG GAG AAG GTG ACA GTG ATC
Leu Gln Ala Gly Ala Asp Ile Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu Val Ala Glu Lys Val Thr Val Ile 150
 H  H  H  C  C  C  H  H  H  H  H  E  E  E  E  C  H  H  H  H  H  H  H  H  H  H  H  H  H  E

ACC AAG CAC AAT GAT GAT GAG CAG TAT GCT TGG GAG TCA TCA GCT GGA GGA TCT TTC ACT GTC AGA CTT GAT AAC GGT GAA CCT TTG GGC
Thr Lys His Asn Asp Asp Glu Gln Tyr Ala Trp Glu Ser Ser Ala Gly Gly Ser Phe Thr Val Arg Leu Asp Asn Gly Glu Pro Leu Gly 180
 E  C  C  C  C  C  H  H  C  H  E  E  C  C  C  C  C  E  E  E  E  E  E  C  C  C  C  C  C  C

CGT GGA ACA AAA GTT ATC CTG CAC CTC AAA GAA GAC CAA ACT GAA TAT CTG GAA GAA CGG CGA ATC AAG GAG ATT GTG AAG AAG CAT TCT
Arg Gly Thr Lys Val Ile Leu His Leu Lys Glu Asp Gln Thr Glu Tyr Leu Glu Glu Arg Arg Ile Lys Glu Ile Val Lys Lys His Ser 210
 C  C  H  E  E  E  E  E  C  C  H  C  C  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  C

CAG TTC ATT GGC TAC CCT ATT AGG CTC TTT GTG GAG AAG GAG CGC GAT AAG GAG GTG AGT GAT GAT GAA GCT GAG GAA AAG GAG GAG GAA
Gln Phe Ile Gly Tyr Pro Ile Arg Leu Phe Val Glu Lys Glu Arg Asp Lys Glu Val Ser Asp Asp Glu Ala Glu Glu Lys Glu Glu Glu 240
 C  H  C  C  C  C  E  H  E  E  E  C  C  C  C  H  C  H  H  H  H  H  H  H  H  H  H  H  H  H

AAA GAG GAG AAG GAG GAG AAG ACA GAA GAT AAA CCA GAG ATT GAG GAC GTT GGT TCT GAT GAG GAA GAG GAA AAG AAG GAT GGA GAT AAG
Lys Glu Glu Lys Glu Glu Lys Thr Glu Asp Lys Pro Glu Ile Glu Asp Val Gly Ser Asp Glu Glu Glu Glu Lys Lys Asp Gly Asp Lys 270
 H  H  H  H  H  H  H  H  H  C  C  C  H  H  C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H

AAG AAG AAA AAG AAG ATC AAG GAG AAG TAC ATT GAT GAG GAA GAG CTC AAC AAG ACC AAG CCT ATT TGG ACC AGG AAC CCA GAT GAC ATC
Lys Lys Lys Lys Ile Lys Glu Lys Tyr Ile Asp Glu Glu Glu Leu Asn Lys Thr Lys Pro Ile Trp Thr Arg Asn Pro Asp Asp Ile 300
 H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  C  C  C  C  E  E  E  E  C  C  C  C  C

ACC AAT GAG GAG TAC GGG GAG TTC TAT AAG AGC CTA ACT AAT GAC TGG GAG GAC CAC TTG GCT GTC AAA CAC TTC TCT GTG GAA GGT CAG
Thr Asn Glu Glu Tyr Gly Glu Phe Tyr Lys Ser Leu Thr Asn Asp Trp Glu Asp His Leu Ala Val Lys His Phe Ser Val Glu Gly Gln 330
 C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H

CTG GAA TTC AGA GCT CTC CTG TTT GTC CCA CGA CGT GCA CCT TTT GAT CTG TTT GAA AAC AGG AAG AAG AAA AAC AAC ATC AAG CTC TAT
Leu Glu Phe Arg Ala Leu Leu Phe Val Pro Arg Arg Ala Pro Phe Asp Leu Phe Glu Asn Arg Lys Lys Lys Asn Asn Ile Lys Leu Tyr 360
 H  H  H  H  H  H  H  H  C  C  C  C  C  H  C  C  H  H  H  H  H  H  C  C  C  C  H  H  H  H

GTA CGC AGA GTT TTC ATC ATG GAC AAC TGT GAG GAA CTG ATC CCC GAA TAC CTG AAC TTC ATG AGA GGT GTC GTA GAC TCT GAG GAT TTA
Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Glu Leu Ile Pro Glu Tyr Leu Asn Phe Met Arg Gly Val Val Asp Ser Glu Asp Leu 390
 E  E  H  E  E  H  H  C  C  C  H  H  C  C  C  H  H  H  H  H  H  C  C  C  H  C  C  C  C  C

CCT CTG AAT ATT TCT CGT GAA ATG CTG CAA CAA AGC AAG ATC CTT AAA GTG ATT CGG AAG AAC TTG GTG AAG AAG TGT TTG GAA CTT TTC
Pro Leu Asn Ile Ser Arg Glu Met Leu Gln Gln Ser Lys Ile Leu Lys Val Ile Arg Lys Asn Leu Val Lys Lys Cys Leu Glu Leu Phe 420
 C  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H

ACT GAG TTG GCT GAA GAC AAG GAG AAC TAC AAA AAG TTC TAT GAG CAG TTC TCC AAG AAC ATC AAG CTT GGA ATA CAT GAA GAC TCC CAG
Thr Glu Leu Ala Glu Asp Lys Glu Asn Tyr Lys Lys Phe Tyr Glu Gln Phe Ser Lys Asn Ile Lys Leu Gly Ile His Glu Asp Ser Gln 450
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AAC CGC AAG AAA CTC TCA GAG TTA CTC AGG TAT TAC ACA TCT GCA TCT GGT GAT GAA ATG GTT TCT CTG AAG GAC TAC TGC ACT CGC ATG
Asn Arg Lys Lys Leu Ser Glu Leu Leu Arg Tyr Tyr Thr Ser Ala Ser Gly Asp Glu Met Val Ser Leu Lys Asp Tyr Cys Thr Arg Met 480
 H  H  H  H  H  H  H  H  H  H  H  C  C  C  C  C  C  C  C  E  E  E  H  C  C  H  H  H  H  H

AAG GAA AAC CAG AAA CAT GTC TAC TAC ATC ACT GGT GAG ACA AAG GAC CAG GTG GCT AAC TCT GCT TTT GTG GAG CGC CTT CGC AAG CAT
Lys Glu Asn Gln Lys His Val Tyr Tyr Ile Thr Gly Glu Thr Lys Asp Gln Val Ala Asn Ser Ala Phe Val Glu Arg Leu Arg Lys His 510
 H  H  C  C  C  C  E  E  E  E  E  C  C  C  C  H  H  H  C  C  H  H  H  H  H  H  H  H  H  C

GGC CTG GAA GTG ATC TAC ATG ATT GAG CCT ATT GAT GAA TAT TGT GTG CAG CAG CTG AAG GAA TTT GAA GGC AAG ACC CTG GTT TCT GTA
Gly Leu Glu Val Ile Tyr Met Ile Glu Pro Ile Asp Glu Tyr Cys Val Gln Gln Leu Lys Glu Phe Glu Gly Lys Thr Leu Val Ser Val 540
 C  H  H  E  E  E  E  E  C  C  C  C  H  H  H  H  H  H  H  H  H  H  C  C  C  H  H  E  H  E

ACA AAA GAG GGC TTA GAG CTT CCA GAA GAT GAA GAA GAG AAG AAG AAA CAG GAG GAG AAA AAA GCC AAG TTT GAA AAC CTT TGC AAA ATT
Thr Lys Glu Gly Leu Glu Leu Pro Glu Asp Glu Glu Glu Lys Lys Lys Gln Glu Glu Lys Lys Ala Lys Phe Glu Asn Leu Cys Lys Ile 570
 E  H  C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H  H

ATG AAA GAT ATC CTT GAG AAG AAA GTA GAA AAG GTT GTT GTG TCC AAT CGC TTG GTA ACT TCT CCA TGC TGT ATT GTA ACA AGT ACA TAT
Met Lys Asp Ile Leu Glu Lys Lys Val Glu Lys Val Val Val Ser Asn Arg Leu Val Thr Ser Pro Cys Cys Ile Val Thr Ser Thr Tyr 600
 H  H  H  H  H  H  H  H  H  H  H  H  H  E  E  C  C  C  C  C  C  C  C  E  E  E  E  E  C  C  C

GGC TGG ACT GCC AAT ATG GAG AGG ATT ATG AAG GCA CAG GCT TTG AGA GAC AAC TCC ACA ATG GGA TAC ATG GCA GCA AAG AAG CAC CTG
Gly Trp Thr Ala Asn Met Glu Arg Ile Met Lys Ala Gln Ala Leu Arg Asp Asn Ser Thr Met Gly Tyr Met Ala Ala Lys Lys His Leu 630
 C  C  H  H  H  H  H  H  H  H  H  H  H  H  C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H

GAG ATC AAT CCT GAT CAT TCC ATC ATT GAA ACA CTG AGG CAG AAG GCA GAG GCT GAT AAG AAT GAC AAA TCT GTG AAG GAT CTT GTC ATA
Glu Ile Asn Pro Asp His Ser Ile Ile Glu Thr Leu Arg Gln Lys Ala Glu Ala Asp Lys Asn Asp Lys Ser Val Lys Asp Leu Val Ile 660
 C  C  C  C  C  C  C  H  H  H  H  H  H  H  H  H  H  H  H  C  C  C  C  H  H  H  H  H  H  H  H

CTG CTG TAC GAG ACA GCT CTC CTG TCC TCT GGC TTT AGT TTA GAA GAT CCC CAG ACA CAT GCC AAC CGC ATT TAC AGA ATG ATC AAA CTT
Leu Leu Tyr Glu Thr Ala Leu Leu Ser Ser Gly Phe Ser Leu Glu Asp Pro Gln Thr His Ala Asn Arg Ile Tyr Arg Met Ile Lys Leu 690
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GGC CTG GGC ATT GAT GAA GAT GAT ACT GCT GCT GAG GAG GCC AGT CCT GCA GTT ACC GAG GAG ATG CCA CCT CTG GAA GGT GAT GAT GAC
Gly Leu Gly Ile Asp Glu Asp Asp Thr Ala Ala Glu Glu Ala Ser Pro Ala Val Thr Glu Glu Met Pro Pro Leu Glu Gly Asp Asp Asp 720
 C  C  C  C  C  C  H  H  H  H  H  H  H  H  C  C  C  H  H  H  C  C  C  C  C  C  C  C  C  C
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ACA TCA CGT ATG GAG GAG GTG GAT TAAAACAGTTTACAGGAACTCATGAATGTTTCCTTGGCTAATATGAATAAGTTATATTTTGTATATTATGAATGTTACCTGCCAAAA
Thr Ser Arg Met Glu Glu Val Asp
 C   C   H   H   H   H   C   C

AAAAACAAAAATCTTTGACACTTTTGTCTGCATTCCCTCTTTATATTTATTTTCAAAGATGTTATACCTTTATTTTTGTTACATTGCTTTTTCAGCTGATGTGAGATACAAATGCCATT

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AAAGTAACAAAGACGGTATGACATCTGGGTGTTCGAGTTTGTTTAGTGTTATACAGCTCTTCAACTCTGAATATGTCTAACATAGAGTACCTAGTAACTAGGTATCTATGAGAGCTGAC

ACTTGGAAGCTTTAACCCTTTTGCTCCTAACAAGGTCTTGATGTTTAAAGTTGTTTTTTATACTGTTCAAGGATTCTGGATTGCACTCTACCATAGAATAGAATCCACTGTAAATCTCT

ATTGTGACTTATGCAAATCTGCATGTACAGTTCAAACCTAGAACTGTAAGAATAAAAGTGTTAAGAATGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAAAA
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Figure 1. Nucleotide and deduced amino acid sequences of chick hsp 90. The protein sequence of 728 amino acids resulting from translation of the 2184 base pair open reading frame is shown beneath the DNA sequence. We sequenced across all subcloning restriction sites. Letters of the third line correspond to the predicted secondary structure (20): H = α helix; C = coil; E = β strand.

sequences for casein kinase II at aa 230 and 259, and four potential sites for N-linked glycosylation at positions 50, 287, 393 and 618 are observed. Hsp 90 phosphorylation has been documented, but no glycosylation has been found so far (3).

The size of the cDNA, ~ 2.9kb, accounts almost completely for the length of the messenger of ~ 3kb detected by Northern blot analysis (9).

The deduced aa chick hsp 90 sequence was compared with the homologous proteins of human (22), mouse (23), drosophila (24), Trypanosoma cruzi (T. cruzi) (25), yeast (26), and E. coli (27). The highest aa identity is observed with human (86%), mouse (87%), and drosophila (80%); with yeast and T. cruzi, the aa identity is of 64% and 66%, respectively. A significant level of identity (44%) is still observed with prokaryotes (E. coli). Two homologous glucose-regulated proteins also are related to the hsp 90 family (28). The human hsp 90α sequence (29 and E. Hickey personal communication) derived from the p801 plasmid (17), used as a probe in this work, exhibits a higher (96%) identity with the chick hsp 90 sequence than the human hsp 90β (22). In addition, near to the N-terminal, the QTQDQP sequence is present in chick, human α, rabbit (6), and in one of the two mouse hsp 90 (30).

Hsp 90 secondary structure prediction. Modelling of the negatively charged A region. Hsp 90 contains two highly charged regions: A between aa 221 and 290, and B between aa 530 and 581,probably exposed to the solvent and involved in protein-protein interaction. They form mostly α-helices, likely stabilized by Glu/Asp and Lys interactions (31). These two regions delineate three separate globular domains where α-helices largely predominates over the β-sheet structures. The secondary structure prediction is shown in Figure 1 (third line).

The dissociation of hsp 90 from the heterooligomeric 8S form of steroid receptors results in a "acidophilic" activation of the hormone binding molecule (32) which acquires DNA-binding properties. Looking for a polyacidic

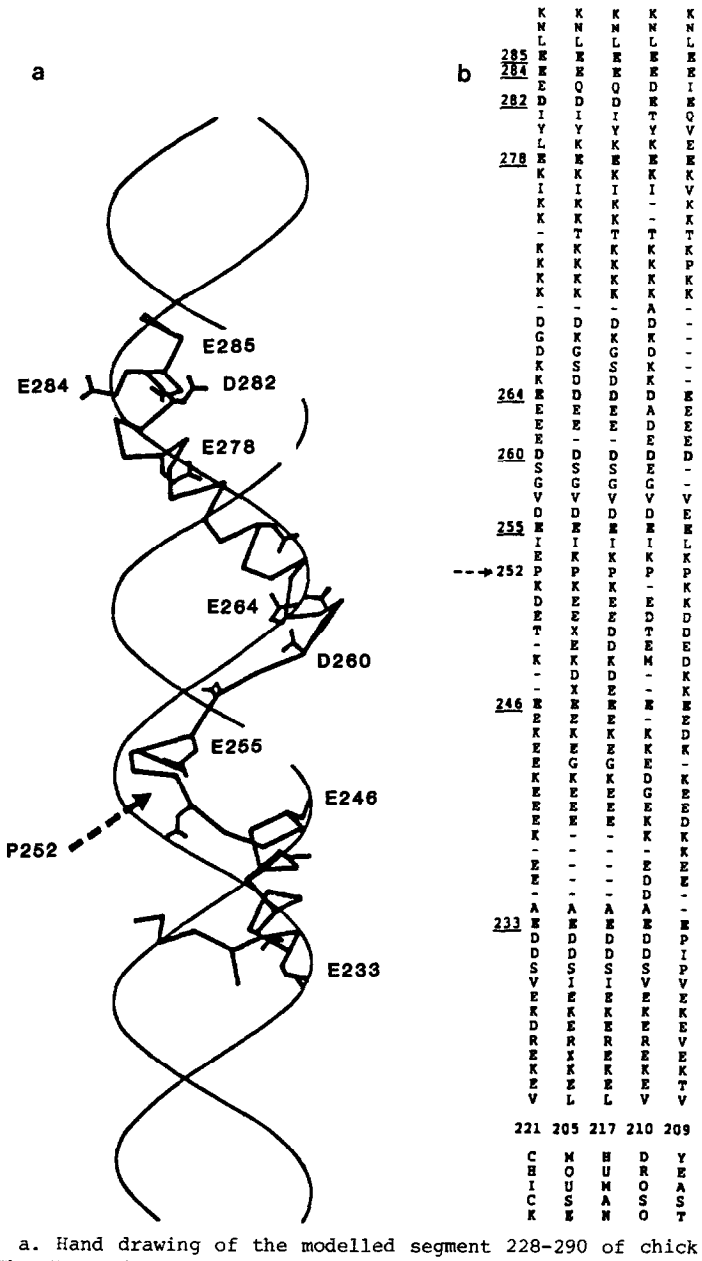

Figure 2. a. Hand drawing of the modelled segment 228-290 of chick hsp 90 A region. The N-terminal segment 221-227, not predicted in α-helix, is not represented. Segment 228-232, predicted with a low probability as helical, was modelled as a coil. The phi and psi angles of the loop region (aa 248-260) were manually adjusted in order to obtain the fitting of a maximum of negatively charged residues to the phosphate backbone of B-DNA structure. During the fitting, the two α-helices (aa 233-247 and aa 261-287) were considered as rigid bodies. Only the α-carbon of the polypeptide backbone and the negatively charged side-chains are represented. b. The numbered residues are the nine most conserved negative amino acids from human to yeast.

stretch of aa which could be involved in hsp 90 receptor interaction, we studied region A which has a large excess of negatively charges aas, mostly Glu. A remarkable topological conservation of the charges in the A region is

observed from human to Drosophila, even though the percentage of amino acid identity between species is lower than in other hsp 90 regions (Figure 2a). In the A region, an α-helix is predicted between aa 233 and 247 and, after a proline-containing loop of 13 aa, a second α-helix is predicted between aa 261 and 287. Similar prediction can be made for hsp90 of all species with the exception of E. coli, where only the first 17 residues of the A region are present and where steroid hormones have not been detected. A long charged α helix of 32 aa has been observed in troponin C by X-ray crystallography (33), thus confirming the previously predicted structure. Modelling of the hsp 90 A region is represented in Figure 2b. The predicted α-helices have been aligned in space along a double helix of B-DNA, superimposing the carboxyl groups of Glu and Asp with the phosphate groups of the DNA backbone, without imposing any constraint to the polypeptide chain predicted structure. Fifteen aa (11 Glu and 4 Asp), out of 30 negatively charged between aa 228 and 290 are aligned with the DNA phosphate groups and nine are conserved from yeast to human. Thus, the A region of hsp 90 may be viewed in space as a DNA-like, polyanionic structure, capable of interaction with the positively charged DNA-binding region of steroid receptors.

DISCUSSION

We report the isolation and the sequence of chick hsp 90 cDNA ; it contains a single open reading frame encoding a protein with a predicted mass of 84,024 Da, a 5' non-coding segment of 19 nt, and a 3' non-coding segment of 632 nt, in agreement with an almost full length cDNA.

Comparison of aa sequence of chick hsp 90 with that of homologous proteins from E. coli to human shows that the hsp 90 family is as highly conserved as the hsp 70 family (1). The chick hsp 90 described here appears to belong to the hsp 90 α-group which is characterized in vertebrates by the sequence QTQDQP near to N terminal (6,30).

The charged regions of protein are candidates for ionic interactions with functionally important macromolecules. In hsp 90, the clustering of acidic aa suggests the possibility of salt bridges with basic aa present in the DNA binding region of steroid receptors. Modelling of hsp 90 A region mimicks DNA configuration, and thus support the hypothesis that, in the 8S form of the receptor, hsp 90 masks the DNA binding domain. Experimental data are in agreement with this hypothesis : the 8S form is reversibly dissociated by salts, and the Zn^{2+} is needed for maintaining both DNA and hsp 90 binding property of the receptor (34,35,36). Antibodies raised against hsp 90 A region or receptor DNA binding region recognised their respective antigen, but not the 8S receptor form (37,38). Experiments with glucocorticosteroid receptor indicate that the steroid binding region also is involved in the formation of

the 8S complex (39). This other interaction may facilitate the ionic binding described here, which we believe determinant in inhibiting the receptor function in absence of hormone, by capping the DNA binding region of steroid receptors.

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