ENZYME ACTIVITIES ASSOCIATED WITH GONADOTROPIC HORMONES

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"Science is always wrong.
It never solves a problem without creating ten more".

George Bernard Shaw

ABSTRACT

A structural relationship between gonadotropic hormones and certain types of enzymes has been suggested in previous studies and an investigation into the possibility of enzymatic activity associated with the gonadotropic hormones has been the primary focus of the research presented in this thesis.

Partial sequence homology between human chorionic gonadotropin (hCG) and α-chymotrypsin prompted the recent proposal of a tertiary structure of hCG using α-chymotrypsin as a folding template, which suggested the possibility of intrinsic peptidase activity associated with hCG. Highly purified hCG (CR127) was assayed for enzymatic activity against a range of synthetic peptide substrates and was found to exhibit Arg-specific peptidase activity. This activity was almost completely inhibited by diisopropylfluorophosphate (DFP), soybean trypsin inhibitor (STI), N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and to a lesser extent by N-α-p-tosyl-L-lysine chloromethyl ketone (TLCK), which indicated that the observed protease activity was serine protease-like. To establish whether this activity was intrinsic to the hormone or due to contaminants, extensive purification procedures were carried out. Hydrophobic interaction chromatography (HIC) and soybean trypsin inhibitor-affinity chromatography were found to effectively separate the protease activity from the hormone, indicating the presence of exogenous protease contaminants in the highly purified preparation of hCG. Further analysis by [^{3}H]-DFP labelling of hCG and SDS-PAGE of the isolated contaminants revealed the presence of possible serine proteases with apparent molecular masses of 60 and 20 kD.

Because serine proteases are known to stimulate cAMP production in the same target cells, it was necessary to determine the effects of the contaminating proteases on the receptor binding of hCG and cAMP production. The presence of these contaminants was found to have no apparent effect on the receptor binding capability of hCG, however the *in vitro* biological activity of hCG as determined by maximal cAMP production was decreased after HIC-HPLC purification of the hormone. These observations suggested that the serine protease-like contaminants contributed to the total cAMP production, thereby introducing significant error in biological assays that use hCG (CR127).

The possible intrinsic enzymatic activity of hCG against its receptor as a natural substrate was further investigated. A membrane-bound receptor preparation was isolated from porcine ovaries and a receptor binding assay successfully established. The effects of hCG

binding upon the membrane-bound receptor were studied and receptor proteolysis was observed. However, this proteolysis could not be definitively attributed to the actions of hCG. A purified receptor was subsequently prepared by hCG-affinity chromatography and analysed by SDS-PAGE with detection by autoradiography and silver staining. The purified receptor was found to have undergone proteolysis during the purification procedure, presumably following incubation with the hCG affinity matrix.

Recent reports of the presence of homologous amino acid sequences in the active site of thioredoxin and the β-subunit of the gonadotropic hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH), and subsequent demonstration of thioredoxin-like activity associated with these hormones, prompted an investigation into the possibility of thioredoxin-like activity associated with hCG. LH, FSH and hCG were all assayed for their ability to promote reactivation of reduced and denatured RNase. Although LH was shown to be capable of reactivating reduced RNase, the level of activity detected was significantly lower than that previously reported, whereas FSH and hCG were not found to be capable of this thioredoxin-like activity. These results suggested that the previously reported thioredoxin-like activity may be due to contamination of the hormone preparation, by the ubiquitous enzyme thioredoxin.

The possibility of LH possessing intrinsic dithiol-disulphide interchange activity was investigated further using [^{3}H]-iodoacetic acid. RNase/LH were incubated in an attempt to quench a dithiol intermediate. Preliminary results suggested that the presence of LH in this reaction increased the amount of protein radiolabelled, however, the isolation of a radiolabelled dithiol intermediate which could be conclusively identified as LH was not forthcoming.

Furthermore the lack of RNase reactivation activity in hCG, suggests that the putative thioredoxin-like activity of LH, if intrinsic, may not be involved in receptor activation and/or signal transduction, as hCG and LH share the same receptor and should therefore have a similar mechanism of activation.

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ABBREVIATIONS

^{1}H-NMR	proton nuclear magnetic resonance
BSA	bovine serum albumin
cAMP	adenosine 3', 5'-cyclic monophosphate
cCMP	cytidine 2':3'-cyclic monophosphate
cpm	counts per minute
DFP	diisopropylfluorophosphate
EDTA	ethylenediaminetetraacetic acid
h	hours
HIC	hydrophobic interaction chromatography
HPLC	high performance liquid chromatography
IAA	iodoacetic acid
kD	kilodaltons
MOPS	3-(N-Morpholino)propanesulfonic acid
PDI	protein disulphide isomerase
PEG	polyethylene glycol
PMSF	phenylmethylsulphonyl fluoride
RNase	ribonuclease A
RP	reverse phase
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
STI	soybean trypsin inhibitor
TEA	triethylamine
TFA	trifluoroacetic acid
TLCK	N-α-p-tosyl-L-lysine chloromethyl ketone
TPCK	N-tosyl-L-phenylalanine chloromethyl ketone
Tris	tris (hydroxymethyl) aminomethane
w/v	weight per volume