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Behaviour of Secretin, Cholecystokinin and Pancreozymin to Oxidation with Hydrogen Peroxide

VIKTOR MUTT

Department of Chemistry II, Karolinska Institutet, Stockholm 60, Sweden

It is known from the work of Dedman, Farmer and Morris that the pituitary adrenocorticotropic hormone may be inactivated by treatment with hydrogen peroxide and the activity restored on reduction, preferably with cysteine.[1] The inactivation is accompanied by the oxidation of the methionine residue of the hormone to the corresponding sulphoxide.[2] An essentially similar behaviour is exhibited by the α- and β- melanocyte stimulating hormones,[3] and by the parathyroid hormone.[4-6]

In work towards the isolation of the gastrointestinal hormones secretin, cholecystokinin and pancreozymin it has been pointed out by Jorpes and Mutt that during the purification procedure it has been easy to separate secretin from cholecystokinin and pancreozymin but that the latter two activities have gone parallel in the various purification steps. This was the case in 1958 when we reported on a preparation with 22 Ivy cholecystokinin units and 120 Crick, Harper and Raper pancreozymin units per mg[7] and it has been true of later work where some one hundred times purer material has been obtained.[8,9] Our purest preparations to date still contain methionine, which is absent from secretin.[10,11] Consequently it seemed to be of interest to determine whether the cholecystokinin and pancreozymin activities would both be affected by mild oxidation with hydrogen peroxide, whether an eventual inactivation would be reversible, and wether secretin, as could be anticipated from the amino acid composition, would exhibit a greater stability to hydrogen peroxide.

It was found in the experiments described below that under conditions of oxidation where secretin loses no activity cholecystokinin is inactivated to the extent of at least 98 %. This inactivation may be largely reversed by treatment of the inactivated material with cysteine. Pancreozymin behaves in this respect like cholecystokinin, although because of the difficult assay methods minor differences in extent of inactivation and reactivation are not excluded by the present investigation.

Table 1. Biological activity of hydrogen peroxide treated secretin, cholecystokinin-pancreozymin, and of the oxidized cholecystokinin-pancreozymin after reactivation with cysteine.

Material	Activity, % of initial
H_2O_2-treated secretin	*ca.* 100
H_2O_2-treated cholecystokinin-pancreozymin	cholecystokinin: < 2 pancreozymin: < 2
H_2O_2-treated cholecystokinin-pancreozymin after reactivation with cysteine	cholecystokinin: *ca.* 90 pancreozymin: *ca.* 90

Experimental. Methods of bioassay. Secretin activity was determined according to Mutt and Söderberg,[12] cholecystokinin according to Ljungberg.[13] For the assay of pancreozymin cats operated as for the assay of secretin were used. The pancreas was induced to secrete a juice of low protein content by the injections of secretin, and the protein expulsed into the juice in response to injections of pancreozymin was determined.

Materials: Secretin; The pure heptacosapeptide,[10,11] was used. *Cholecystokinin-pancreozymin;* this was prepared essentially as described in 1961.[14] *Peroxide solution;* 1 ml of a 30 % solution of hydrogen peroxide ("Perhydrol", Merck, Darmstadt, Germany) was made up to 100 ml with 0.05 M AcOH.

Oxidation. Each hormone preparation was dissolved in the peroxide solution to a concentration of 1 mg/ml and allowed to stand at room temperature for 45 min. The solutions were then diluted with five volumes of distilled water and lyophilized. The lyophilized materials were bioassayed. The results are shown in Table 1. It is evident that whereas secretin has lost no activity both cholecystokinin and pancreozymin have been inactivated to the extent of at least 98 %. Under much more drastic conditions secretin too is known to be inactivated by hydrogen peroxide.[15]

Reactivation of cholecystokinin-pancreozymin. The inactivated material was dissolved to a concentration of 2 mg/ml in 50 % (w/v) aqueous cysteine hydrochloride. The solutions were stored at room temperature for 72 h in an atmosphere of argon. Appropriate dilutions were then made for the assay. Cysteine in the amounts that contaminated the hormone preparations in these dilutions shows no activity in the assay systems used. The results of the assay are given in Table 1. There was an approximately 90 % recovery of both activities.

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On the Resolution of Absorption Spectra

BJÖRN ROOS

Institute for Theoretical Physics, University of Stockholm, Stockholm, Sweden

An electronic absorption spectrum consists in general of a number of overlapping absorption bands. The simple way of interpreting these spectra, is to determine the different absorption peaks by means of the maxima and inflexions on the spectra. This method does not in any way take care of all the information the spectra can give. It only gives an, in some cases very uncertain, estimate of the wavenumber. Bands that are hidden behind other bands with larger extinction coefficients are not detected at all. If one is interested to study small shifts in absorption spectra for a number of near lying complexes, this spectacular method is very risky since shift of one peak on the spectrum