PREPARATION AND PROPERTIES OF THE o-NITROPHENYL SULFENYL DERIVATIVE OF ACTH: AN INHIBITOR OF THE LIPOLYTIC ACTION OF THE HORMONE

by

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Summary. Chemical modification of the single tryptophan residue in adrenocorticotropin (ACTH) by reaction with o-nitrophenyl sulfenyl chloride results in the loss of the lipolytic activity of the hormone. The lipolytic action of unmodified ACTH is blocked by the o-nitrophenyl sulfenyl derivative. The inhibitor has no effect on the action of glucagon on isolated fat cells.

In the course of studies of the structure-activity relationships of adrenocorticotropin (ACTH), we have prepared a number of derivatives of the hormone by selective chemical modification of the side chains of one or more amino acid residues. In this communication we wish to report the preparation and properties of o-nitrophenyl sulfenyl ACTH (NPS-ACTH) in which the single tryptophan residue was modified. The ability of ACTH to stimulate lipolysis in isolated fat cells was found to be completely inhibited by NPS-ACTH.

Materials and Methods.

o-Nitrophenyl sulfenyl chloride was purchased from Eastman Organic Chemicals; Glucagon from Mann Research Laboratories; Tripalmitin from Calbiochem.

Fat cells were prepared from the epididymal fat pads of Sprague-Dawley rats (250-300 g) according to the procedure of Rodbell (1). The cells were suspended in Krebs-Ringer bicarbonate buffer containing 4% bovine serum albumin to yield a suspension containing 30-40 micromoles of triglyceride per ml. Aliquots of 0.5 ml of the cell suspension were incubated at 37° with 0.3 ml Krebs-Ringer bicarbonate buffer and 0.2 ml of hormone (or 0.2 ml water in the case of controls) giving a final volume of 1 ml. All incubations were performed in quadruplicate except the controls which were run in duplicate. Glycerol was determined according to the method of Korn (2). The triglyceride content of the adipocytes was measured by the procedure of Rapport and Alonzo (3) using tripalmitin as standard. The results are expressed as micromoles of glycerol produced per millimole of triglyceride per 2 hr.

Preparation of NPS-ACTH.

Twenty five mg α_s-ACTH (4) was dissolved in 0.2 ml water and then 1.8 ml glacial acetic acid was added. o-Nitrophenyl sulfenyl chloride (25 mg) was added and the reaction mixture kept at room temperature for 3 hr. with occasional shaking. The product was precipitated by the addition of 10 ml ethyl acetate. The supernatant was removed and extracted with 10 cc 0.1N acetic acid. The precipitate was dissolved in the aqueous phase and lyophilized to yield 30 mg yellow fluffy material. This was purified by chromatography on a 25 x 1 cm column of carboxy methyl cellulose (CMC) using a gradient of ammonium acetate as described for ACTH (4). o-Nitrophenyl sulfenyl ACTH (NPS-ACTH) emerged from the column when the concentration of ammonium acetate in the effluent reached a value of 0.185 M. ACTH was found to emerge from the same column at

an effluent ammonium acetate concentration of 0.17 M. NPS-ACTH was rechromatographed on CMC and found to be homogeneous. Electrophoresis on Whatman No. 1 paper in 5% acetic acid (pH 2.6, 10 v/cm) for 5 hr. revealed a single yellow, ninhydrin positive, Ehrlich negative spot with a mobility of 0.67 x lysine. The amino acid composition of an acid hydrolysate of NPS-ACTH was found to be in excellent agreement with that of ACTH. The ultraviolet absorption spectrum of NPS-ACTH in 0.001 N HCl exhibited maxima at 281 mμ (ϵ = 18600) and 365 mμ (ϵ = 4000).

Results and Discussion.

The use of o-nitrophenyl sulfenyl chloride for the selective modification of the indole ring of tryptophan residues in peptides and proteins was first reported by Scoffone et al. (5). In their study, a tetracosapeptide corresponding to the first 24 residues of ACTH (6) was used as a model for investigating the sulfenylation reaction. The biological properties of the modified tetracosapeptide, however, have not been reported until now. We prepared the NPS derivative of ACTH in order to assess the importance of the tryptophan residue in the biological actions of this hormone and to see if it would serve as an antagonist to ACTH.

It can be seen from the results shown in Table I that the modification of the tryptophan residue of ACTH by reaction with o-nitrophenyl sulfenyl chloride leads to a total loss of lipolytic activity. Whereas ACTH stimulates the production of glycerol significantly at a concentration of 7×10^{-9} g/ml, NPS-ACTH is inactive even at a concentration of 1.2×10^{-6} g/ml. Higher levels of NPS-ACTH (up to 5×10^{-5} g/ml) failed to show any lipolytic activity. NPS-ACTH is seen to be an effective inhibitor of ACTH when the concentration of NPS-ACTH is 100 times that of the hormone. Complete

Table I

Inhibition of the Lipolytic Activity of ACTH by NPS-ACTH

Concentration of ACTH μg/ml	Glycerol Production	
	without NPS-ACTH	with NPS-ACTH (1.2 μg/ml)
	μmoles/mmole triglyceride/2 hr.	
0	0.5	4.2 ± 0.5
	1.4	2.5 ± 0.4
0.0035	18.2 ± 0.3	-
	24.9 ± 1.3	-
0.007	35.4 ± 1.1	5.9 ± 0.6
	40.5 ± 1.5	5.4 ± 1.2
0.014	60.2 ± 1.0	6.1 ± 0.4
	78.3 ± 0.7	5.1 ± 0.2

Values are the mean ± S. E. for two representative experiments.

inhibition of the action of ACTH on adipocytes has been observed at an ACTH : Inhibitor ratio of 1 : 25. These results suggest that the tryptophan residue of ACTH may be functionally essential for the stimulation of lipolysis in isolated adipocytes. It is also apparent that modification of the tryptophan residue does not prevent the binding of the hormone to its receptor.

Recently, Birnbaumer and Rodbell (7) have reported that a synthetic analog of the tetracosapeptide corresponding to the first twenty four residues

Table II

Effect of NPS-ACTH on the Lipolytic Activity of Glucagon

Concentration of Glucagon μg/ml	Glycerol Production	
	without NPS-ACTH	with NPS-ACTH (5.8 μg/ml)
	μmoles/mmole triglyceride/2 hr.	
0	0.8	3.9 ± 0.4
	1.0	4.0 ± 0.3
0.05	5.1 ± 1.3	10.1 ± 0.4
	3.2 ± 0.4	6.2 ± 1.4
0.25	10.8 ± 0.5	16.0 ± 0.6
	10.4 ± 0.4	15.0 ± 0.7

of ACTH (containing D-Glu-D-His-D-Phe-D-Arg-D-Trp in place of L-Glu-L-His-L-Phe-L-Arg-L-Trp) is inactive and able to inhibit the action of ACTH on isolated fat cells. The results presented here show that the modification of the single tryptophan residue by reaction with o-nitrophenyl sulfenyl chloride is sufficient for inactivation of the hormone. NPS-ACTH which is also an effective inhibitor of ACTH can be prepared much more readily by the simple procedure described above.

The specificity of the inhibitory action of NPS-ACTH is demonstrated by its inability to block the lipolytic action of glucagon (Table II). The presence of a high concentration of NPS-ACTH (5.8×10^{-6} g/ml) does not impair the ability of glucagon to stimulate glycerol production. This

result supports and confirms the observations of Birnbaumer and Rodbell (7) that even though a number of structurally dissimiliar hormones appear to stimulate lipolysis in isolated fat cells by a common action (via the stimulation of adenyl cyclase), the various hormones act at discrete sites which are specific for each hormone. The effect of NPS-ACTH on the stimulation of the adenyl cyclases of different tissues by ACTH and other agents is under investigation.

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