Degradation of somatomedins by the thioredoxin system

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The insulin disulfide reducing thioredoxin system from *E.coli* was used to investigate a possible mechanism of degradation for the two somatomedins, insulin-like growth factor I and II (IGF-I and -II). The amounts of IGF-I and -II remaining after degradation were measured by use of human placenta radioreceptor assay. The results show that both IGF-I and -II were as sensitive to disulfide reduction as insulin.

Insulin *Degradation* *Thioredoxin* *Insulin-like growth factor I* *Insulin-like growth factor II*

1. INTRODUCTION

The somatomedins are a family of polypeptide hormones with growth-stimulating and insulin-like activities in vitro [1–3]. Insulin-like growth factor I (IGF-I) and II (IGF-II), two members of the somatomedin family purified to homogeniety from human plasma, are low molecular mass peptides of 7.5 kDa [4,5]. Both have a high degree of sequence homology to proinsulin. This sequence homology which includes 3 disulfide bridges also present in insulin has been used to predict conformational models of IGF-I and -II based on the known structure of insulin [6].

The reduced form of thioredoxin [thioredoxin-$(SH)_2$] is an efficient reductant of certain exposed protein disulfides [7–9]. The disulfide bridges of insulin and proinsulin are efficiently reduced in vitro by thioredoxin-$(SH)_2$ from rat liver and *Escherichia coli* [7–9]. The physiological importance of this process is still unclear, but one hypothesis is that the first step in degradation of insulin in vivo, is an enzymatic reduction of disulfide bonds followed by proteolytic degradation of free A and B chains [10,11]. It has been suggested that the degradation of insulin occurs after binding to its receptor, followed by internalization of the receptor-hormone complex [12].

To elucidate a possible mechanism of degradation, and to study the reactivity of disulfide bonds in IGF-I and -II, we have used the thioredoxin system. The results show that both IGF-I and -II were degraded by thioredoxin-$(SH)_2$ with the same or higher efficiency as insulin.

2. MATERIALS AND METHODS

2.1. *Materials*

Thioredoxin and thioredoxin reductase were prepared from *E. coli* B as previously described in [13,14]. IGF-I and -II were purified to homogeneity from human plasma according to the method of Enberg et al. [15].

Porcine insulin was obtained from Novo A/S (Denmark) and NADPH from Sigma (MO, USA). All other chemicals were of laboratory analytical grade.

2.2. *Reduction and alkylation*

A reaction buffer composed of 0.05 M Tris, pH 7.4, 0.2% human serum albumin and 1 mM EDTA was used for all dilutions. Stock solutions of 0.8 mM NADPH, 500 μM thioredoxin, 10 μM thioredoxin reductase and 5 mM iodoacetic acid were used.

A reaction mixture was prepared fresh from 500 μm reaction bufferm 2 μl or 500 μM thioredoxin and 10 μl of 10 μM thioredoxin reductase.

The degradation studies were performed at room temperature. To 200 or 500 ng pure peptide

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(lyophilized in siliconized glass tubes) 60 μl of reaction buffer was added. Ten μl of 0.8 mM NADPH was added and a control sample of 10 μl was taken at time 0. The reaction was started by addition of 10 μl reaction mixture and 10-μl samples were taken at the specified times. The reactions were terminated by addition of 5 μl of 5 mM iodoacetic acid followed by 1 h incubation at room temperature.

2.3. *Radioreceptor assays*

Labelling of insulin, IGF-I and II with ^{125}I was done with the lactoperoxidase method, followed by separation of the labelled peptides from ^{125}I by ion-exchange chromatography on CM-cellulose as in [16]. A radioreceptor assay utilizing human placental membranes as matrix, was used to measure the amount of non-degraded material [17]. All values are expressed as percent of the amount of peptide originally present in the starting material.

3. RESULTS

The effect of disulfide reduction of insulin on its receptor binding was tested by incubation of 0.4–0.8 μM insulin with thioredoxin and thioredoxin reductase in the presence of NADPH. To inactivate completely the thioredoxin system the reaction was stopped by alkylation of nascent sulfhydryl groups by addition of excess iodoacetic acid. As seen from table 1, the receptor binding activity of insulin was rapidly lost upon incubation, which is consistent with reduction and alkylation of essential disulfide bonds in the molecule. Control samples exposed only to excess iodoacetic acid showed full receptor binding activity, excluding any detrimental effect of iodoacetic acid.

Table 1

Results of degradation by the thioredoxin system

min	IGF-I		IGF-II		Insulin	
0	96.6	11.6	94.3	2.3	102.0	5.0
0.5	nd		73.5	28.1	nd	
1	39.7	2.7	38.3	5.5	51.3	8.1
2	27.9	8.7	21.7	1.5	42.7	3.4
5	10.7	0.8	7.8	2.3	9.3	0.5

The values are expressed in percent of the peptide originally present in the starting material. Each value represents the mean ± SD of 3 experiments. nd, not determined

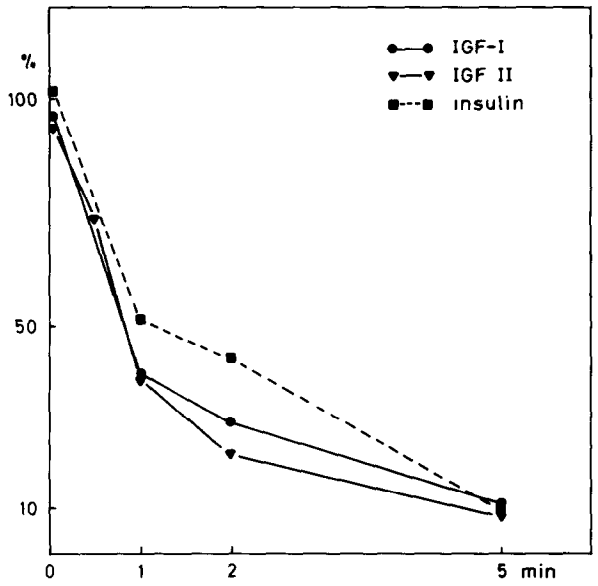

Fig.1. Rate of degradation for insulin (■—■), pure human IGF-I (●—●) and IGF-II (▼—▼) at concentrations between 0.4 and 0.8 μM, by the thioredoxin system. Thioredoxin was prepared from *E. coli*. The amount of non-degraded material was measured by human placenta radioreceptor assay. The values are expressed as percent of the peptide originally present in the starting material. Each point represents the mean of 3 experiments.

Incubations of either IGF-I or -II with the thioredoxin system at similar concentrations to insulin, also gave a rapid degradation of both peptides (table 1). The rate of degradation was similar for both IGF-I and -II, and identical to or higher than that for insulin (fig.1). These results are consistent with essential reactive disulfides in both IGF-I and -II, and as for insulin, no decreased binding of IGF-I and -II exposed only to iodoacetic acid was observed.

4. DISCUSSION

The degradation of insulin in vivo occurs mainly in the liver and kidney, while the remainder is degraded by peripheral tissue [18]. At the cellular level 3 enzyme systems have been investigated, insulin protease, glutathione insulin transhydrogen-

ase (GIT) and lysosomal enzymes. It has been shown that a prerequisite for the degradation of insulin is receptor binding, and an internalization of the receptor-hormone complex [18].

Based on the conformational models of IGF-I and -II by Blundell et al., we assumed that the insulin disulfide reducing thioredoxin system, might be used to study a possible mechanism of degradation for the somatomedins in vitro. Our results show that receptor binding of IGF-I and -II requires intact disulfide bridges. Furthermore the results demonstrate that the reactivity of disulfides in IGF-I, IGF-II and insulin are similar, using thioredoxin-$(SH)_2$ as a probe. This is in accordance with the predicted conformational models [6]. The number of disulfides in IGF-I and -II reacting with thioredoxin-$(SH)_2$ were not determined here due to the limited amounts of pure material available. Instead, insulin with its well characterized reactivity with thioredoxin [7] was used for comparison. When larger amounts of IGF-I and -II are available, a quantitation and identification of the reactive disulfides by titration of the nascent sulfhydryl groups with radioactively labeled iodoacetic acid will be possible.

In previous investigations utilizing different enzyme preparations from rat liver [19], rat kidney [20], rat skeletal muscle [21] and human leucocytes [22], the rate of degradation of somatomedins has been compared to insulin. The results show that both IGF-I and -II could be degraded by the same enzyme systems as insulin. The rate of degradation, however, was less than that for insulin. This is in contrast to our results, in which both IGF-I and -II were degraded with the same or higher potency as insulin.

In conclusion, this study shows that the disulfide reducing enzyme thioredoxin, normally present in mammalian cells [23,24], degrades both IGF-I and -II under physiological conditions. Whether this system has any physiological importance in vivo, will require further studies.

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