

# Evaluation of the metal ion requirement of the human deoxyhypusine hydroxylase from HeLa cells using a novel enzyme assay

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Received 22 November 1995

**Abstract** Hypusine synthesis in the eukaryotic initiation factor 5A is a unique two-step posttranslational modification. After deoxyhypusine is generated by the deoxyhypusine synthase, the deoxyhypusine hydroxylase (EC 1.14.99.29) catalyzes the formation of mature hypusine. A rapid assay for monitoring the deoxyhypusine hydroxylase activity was established, employing the oxidative cleavage of the hypusyl residue and subsequent extraction of the generated aldehydes. As metal ion chelators have been reported to inhibit the deoxyhypusine hydroxylase, the mechanism of this inhibition and the effect of transition metal ions on the enzyme activity were investigated. A ferric ion appears to be essential for enzymatic activity, the inhibition of which is entirely attributed to the metal ion binding capacity of the chelators.

**Key words:** Eukaryotic initiation factor 5A; Hypusine; Posttranslational modification; Mimosine; Ciclopirox olamine; Metal chelation

## 1. Introduction

Eukaryotic initiation factor 5A (eIF-5A) with its unique amino acid hypusine is highly conserved in all eukaryotes and in archaeobacteria, but could not be found in eubacteria. Even though the precise in vivo function of this protein is still unknown, hypusine formation in eIF-5A was shown to be central to cell viability [1–3].

The posttranslational modification which leads to the biosynthesis of the amino acid hypusine in eIF-5A is a two-step process. In the first step, the aminobutyl moiety of spermidine is transferred to the  $\epsilon$ -amino group of a specific lysyl residue (at amino acid position 50 in human eIF-5A) in an NAD<sup>+</sup>-dependent manner. This reaction is catalyzed by the enzyme deoxyhypusine synthase. Further modification of the resulting deoxyhypusine intermediate by the deoxyhypusine hydroxylase generates the mature hypusine-containing eIF-5A (for review see [4]). The deoxyhypusine synthase from *Neurospora crassa* [5], rat [6], and man [7] was recently purified and biochemically

characterized and the genomic DNA sequence encoding the deoxyhypusine synthase in *Saccharomyces cerevisiae* has been identified [7,8]. cDNA cloning and expression of the recombinant human deoxyhypusine synthase was published recently [9,10].

However, little is known about the second enzyme of the hypusine biosynthesis pathway deoxyhypusine hydroxylase. Inhibition studies indicated that this enzyme can be inactivated by both metal chelators [11,12] and polyamines [13]. Furthermore, the inhibition of the deoxyhypusine hydroxylase *in vivo* correlates with cell cycle arrest at the G1/S boundary [14,15].

Most studies on deoxyhypusine hydroxylase activity *in vitro* [12,13] and *in vivo* [14–16] made use of amino acid composition analysis [2,17], which allows the distinction between deoxyhypusine and hypusine. Another method for discriminating between the two modification states is based on the specific oxidation of the hypusyl residue [18]. In the work presented here, a novel and rapid *in vitro* assay for monitoring the deoxyhypusine hydroxylase activity is described. This assay is based on mild oxidation of the product, i.e. the hypusine residue [19] and subsequent selective extraction of the generated aldehydes with organic solvent [20]. Using this assay, we were able to analyze the mode of inhibition of the metal chelators and the specificity of the metal ion dependency of the human deoxyhypusine hydroxylase from HeLa cells *in vitro*.

## 2. Materials and methods

### 2.1. Materials

HeLa S3 cells were purchased from the Computer Cell Culture Centre, University of Mons, Belgium. Chromatography materials and columns were purchased from Pharmacia, Freiburg, Germany. [<sup>14</sup>C]Spermidine trihydrochloride (*N*-(3-aminopropyl)-[1,4-<sup>14</sup>C]tetramethylene-1,4-diamine trihydrochloride) with a specific activity of 114 mCi/mmol was from Amersham Int. plc, Little Chalfont, England, UK. [<sup>3</sup>H]Spermidine trihydrochloride ([terminal methylenes-<sup>3</sup>H](*N*-(3-aminopropyl)-tetramethylene-1,4-diamine trihydrochloride) with a specific activity of 16.4 Ci/mmol was obtained from DuPont NEN, Dreieich, Germany. Metipirox and the derivative of Ciclopiroxolamine (CPX), 4,6-diphenyl-1-hydroxy-pyridine-2-one (DHP), were synthesized as previously published [21]. CPX, mimosine,  $\alpha,\alpha'$ -dipyridyl, sodium metaperiodate,  $\delta$ -hydroxylysine hydrochloride, and dimedone(5,5-dimethyl-1,3-cyclo-hexanedione) were from Sigma Chemical Co., St. Louis, USA, spermidine (non-radioactive) was from Calbiochem, and NAD<sup>+</sup> from Boehringer-Mannheim Corp., Germany. All other reagents were of analytical grade.

### 2.2. Preparation of deoxyhypusine-containing eIF-5A substrate

In order to generate radioactively labeled deoxyhypusine-containing eIF-5A as substrate for the deoxyhypusine hydroxylase assay, purified recombinant eIF-5A precursor protein [22] was enzymatically modified. With minor modifications, this reaction was carried out as described previously, using the human deoxyhypusine synthase (spermidine

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**Abbreviations:** CPX, ciclopirox olamine; deoxyhypusine synthase, spermidine dehydrogenase (NAD<sup>+</sup>, 1-deoxyhypusyl-forming and aminobutyl-transferring); EDTA, (ethylenedinitrilo)tetraacetic acid; DHP, 4,6-diphenyl-1-hydroxy-pyridine-2-one; eIF-5A, eukaryotic initiation factor 5A.

ine dehydrogenase, NAD<sup>+</sup>, 1-deoxyhypusyl-forming and aminobutyl-transferring) purified from HeLa cells [23]. The standard reaction mixture contained either 44  $\mu$ M [<sup>14</sup>C]spermidine or [<sup>3</sup>H]spermidine (diluted with non-radioactive spermidine to a specific activity of 440 mCi/mmol), 2.5 mM NAD<sup>+</sup>, 3  $\mu$ M recombinant eIF-5A precursor protein and 6 units of deoxyhypusine synthase in 0.3 M glycine/NaOH buffer, pH 9.0, 1 mM DTT and was incubated for 3 h at 37°C. (One unit is defined as the amount of enzyme which catalyzes the formation of 1 pmol of product per 3 h under the given condition). For separation of labeled eIF-5A from excess spermidine, 300  $\mu$ l aliquots of the reaction mixture were passed over Sephadex G-25 M gel filtration columns (PD-10 columns, Pharmacia), which were equilibrated with 50 mM sodium phosphate buffer, pH 7.5, 6 mM DTT. After measuring the incorporated radioactivity in a liquid scintillation counter, aliquots containing 22,500 cpm of labeled eIF-5A were frozen at -20°C. Approximately 70% of the eIF-5A molecules are modified to their deoxyhypusine-containing form as judged by mass spectrometry.

### 2.3. Deoxyhypusine hydroxylase assay

The deoxyhypusine hydroxylase uses deoxyhypusine-containing eIF-5A as its natural substrate to form hypusine-modified eIF-5A. This product can be oxidized to yield the free lysine form of eIF-5A (precursor), formaldehyde and  $\beta$ -aminopropionaldehyde [18]. Following oxidative cleavage, the generated radioactively labeled aldehydes can be specifically converted with dimedone, and extracted into the organic phase in analogy to the methods previously described for hydroxypropyl and hydroxylslyl residues [20].

HeLa cell extracts were prepared as previously described [23]. Briefly, 35 g of HeLa cells were resuspended in 280 ml of 10 mM Tris-HCl, pH 7.0, 10 mM NaCl, 5 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10  $\mu$ g/ml aprotinin, and 10  $\mu$ g/ml leupeptin, and disrupted in a Dounce homogenizer. After centrifugation at 40,000  $\times$  g for 10 min at 4°C the clear supernatant was frozen in liquid nitrogen.

250  $\mu$ l of HeLa cell extract were incubated at 37°C for 3 h in 50 mM sodium phosphate buffer, pH 7.5, containing 6 mM DTT and 22,500 cpm of labeled deoxyhypusine-containing eIF-5A ( $\approx$  80 nm). All steps following this hydroxylase reaction were conducted at room temperature. After diluting the sample with 2 vols. of 45 mM citric acid and 210 mM Na<sub>2</sub>HPO<sub>4</sub>,  $\delta$ -hydroxylysine hydrochloride was added as a carrier to a concentration of 1.25 mM. The oxidation of the hydroxylated amino acids to their aldehyde forms was then carried out using sodium meta-periodate at a final concentration of 50 mM. Upon addition of dimedone (5,5-dimethyl-1,3-cyclo-hexanedione) to a final concentration of 80 mM, the generated aldehydes were converted into toluene-soluble compounds. Following extraction of the modified aldehydes with one volume of toluene by vigorous shaking for 30 min and subsequent centrifugation for 10 min at 1500  $\times$  g, the radioactivity in the organic phase was measured in a liquid scintillation counter.

### 2.4. Spectrophotometric competition titration

The EDTA-Fe(III) complex was formed by incubating 30  $\mu$ M Fe(NO<sub>3</sub>)<sub>3</sub> in phosphate-buffered saline (10 mM sodium phosphate at pH 7.2, 150 mM NaCl) at 37°C for 16 h with various concentrations of EDTA (1  $\mu$ M–400 mM). After CPX, metipirox, or DPHP, respectively, were added to a final concentration of 100  $\mu$ M, incubation was continued for 3 h. Following an extraction with ethylacetate, the absorption of the organic phase was determined in a Beckman DU-65 spectrophotometer at 410 nm. For the titration of the EDTA-Fe(III) complex with mimosine, 10-fold higher concentrations of the EDTA-Fe(III) complex were used and the absorption of the aqueous phase was measured at 445 nm. The relative  $K_d$  value for  $\alpha,\alpha$ -dipyridyl was determined by titration of its Fe(III) complex with mimosine. Based on these titration curves the dissociation constant for the ligand from Fe(III) in relation to the known dissociation constant for the competitor EDTA from Fe(III) was calculated. The  $K_d$  for the reaction [3 ligand + Fe(III)  $\rightleftharpoons$  (ligand)<sub>3</sub>Fe(III)] determined by the algebraically correct description of the binding equilibria in a mixture of competing ligands [24] extended with the possibility for variable stoichiometries between the ligands and substrate, is the intrinsic site dissociation constant for the case of the ligand binding to 3 equivalent non-interacting sites on Fe(III). The resulting  $K_d$  values for the different chelators show that the estimation of equivalent extinction coefficients for 3 (ligand)<sub>3</sub>Fe(III) complexes provides an appropriate degree of sensitivity to structural variations between the ligands.

## 3. Results and discussion

The oxidative cleavage of the hypusyl residue allows its specific distinction from the deoxyhypusyl residue [18]. Harsh oxidation conditions using permanganate and periodate leads to the formation of the lysine form of eIF-5A, formic acid and  $\beta$ -alanine. However, formaldehyde and  $\beta$ -aminopropionaldehyde are produced instead of the carbonic acids, if only periodate is employed in the oxidation [19]. These aldehydes can be specifically extracted with toluene following their incorporation into dimedone dimers [20]. Based on the oxidation of the radioactively labeled hypusyl residue and subsequent extraction of the generated aldehydes, a rapid assay for measuring the deoxyhypusine hydroxylase activity has been developed. The specificity of this assay is proven by the following control experiments. (1) In the absence of the deoxyhypusine hydroxylase containing cell extract, no radioactivity above background was found in the toluene phase (Table 1), which confirms the requirement for the hydroxyl group for the oxidative cleavage reaction and the specificity of the oxidation for a mature hypusyl residue. (2) No radioactivity above background was found in the toluene phase, in the case where NaIO<sub>4</sub> was omitted from the oxidation reaction. (3) Measurable hydroxylase activity is only observed in the presence of the substrate deoxyhypusine-containing eIF-5A. Equivalent amounts of free labeled spermidine plus unmodified precursor eIF-5A resulted in the extraction of only minute amounts of radioactivity into

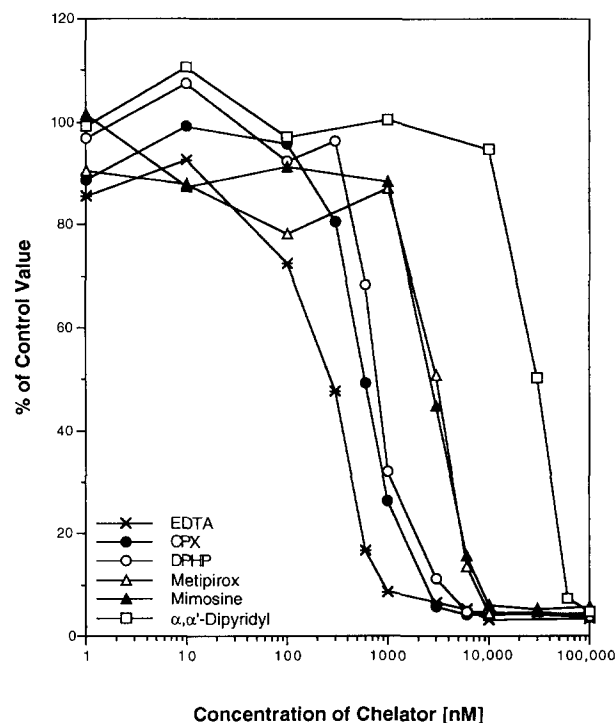


Fig. 1. Concentration-dependent inhibition of the deoxyhypusine hydroxylase by various metal chelators. EDTA, mimosine, and  $\alpha,\alpha$ -dipyridyl were dissolved in PBS; CPX, metipirox, and DPHP were dissolved in 40% ethanol. Corresponding amounts of the chelators were added to the cell extracts prior to incubation with the [<sup>3</sup>H]deoxyhypusine-containing substrate. The hydroxylase reaction was performed in 50 mM potassium phosphate, pH 7.5, containing 6 mM DTT for 3 h. Determination of the amount of newly formed [<sup>3</sup>H]hypusine was done by oxidation with NaIO<sub>4</sub>, dimedone conversion of the oxidation products and subsequent extraction with toluene.

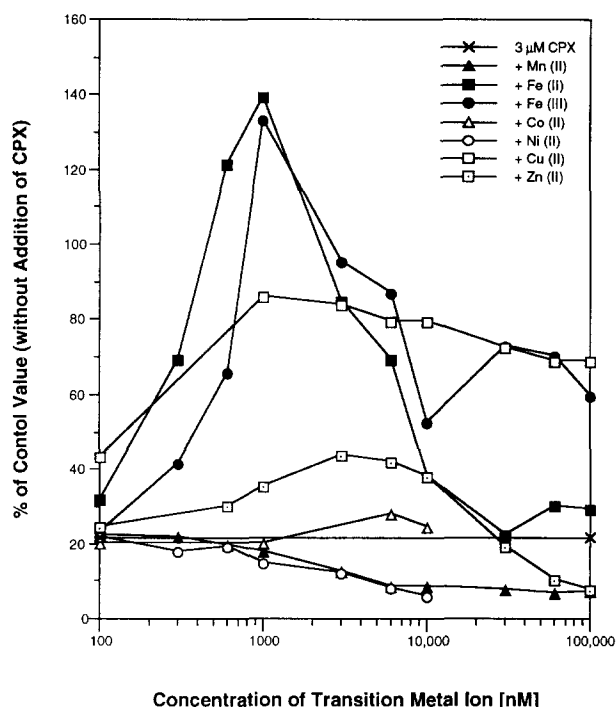


Fig. 2. Reconstitution of the deoxyhypusine hydroxylase by various transition metal ions in the presence of CPX. 3  $\mu$ M CPX plus the indicated concentrations of the respective transition metal ions were added to the cell extracts prior to substrate incubation. The enzyme reaction and product determination were performed as outlined in the legend to Fig. 1. The residual enzyme activity after inhibition with 3  $\mu$ M CPX alone is indicated by the straight line in the lower part of the graph. All values are given in percent of the control value, i.e. without addition of CPX or metal ion.

the toluene phase. The observed 3-fold background activity might be explained by either a weak hydroxylation and subsequent oxidation of spermidine itself or by a slow formation of labeled hypusine from the unmodified eIF-5A precursor, since crude cell extracts containing both the deoxyhypusine synthase and the deoxyhypusine hydroxylase were used.

Whereas the use of [ $^{14}$ C]deoxyhypusine-containing substrate in the hydroxylase reaction results in the formation of both  $^{14}$ C-labeled formaldehyde and  $^{14}$ C-labeled  $\beta$ -aminopropionaldehyde during the oxidation of the mature hypusyl residue, the radioactive label is only incorporated into the  $\beta$ -aminopropion-

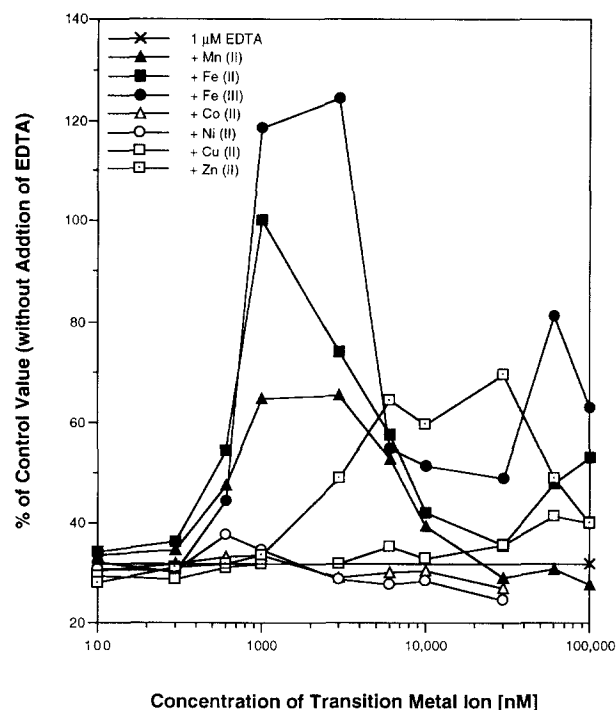


Fig. 3. Reconstitution of the deoxyhypusine hydroxylase by various transition metal ions in the presence of EDTA. 1  $\mu$ M EDTA plus the indicated concentrations of the respective transition metal ions were added to the cell extracts prior to substrate incubation. The enzyme reaction and product determination were performed as outlined in the legend to Fig. 1. The residual enzyme activity after inhibition with 1  $\mu$ M EDTA alone is indicated by the straight line in the lower part of the graph. All values are given in percent of the control value, i.e. without addition of EDTA or metal ion.

aldehyde oxidation product, if [ $^3$ H]deoxyhypusine-containing eIF-5A is used as substrate. Both aldehydes can be toluene extracted after conversion with dimedone, since both substrates, carrying either the  $^{14}$ C- or the  $^3$ H-label, give comparable results in the hydroxylase assay (Table 1). Moreover, a signal-to-noise ratio of 100:1 and a standard deviation of less than 5% was observed for either of the assay systems. The specific extraction of the dimedone converted aldehydes into the toluene phase as the final step in this assay allows a convenient and fast analysis of the hypusine content of the test samples. The time- and protein-consuming hydrolysis of eIF-5A and subsequent

Table 1  
Comparison of  $^{14}$ C-labeled versus  $^3$ H-labeled substrate for monitoring the deoxyhypusine hydroxylase activity

	$^{14}$ C]Assay [mean dpm value] ( $\pm \sigma$ ) <i>n</i> = 3	$^3$ H]Assay [mean dpm value] ( $\pm \sigma$ ) <i>n</i> = 3	% of control [ $^{14}$ C]/[ $^3$ H]
Control	2,045 ( $\pm$ 78)	2,394 ( $\pm$ 122)	100/100
(1) Incubation without cell extract <sup>a</sup>	23 ( $\pm$ 4)	19 ( $\pm$ 7)	1.1/0.8
(2) No NaIO <sub>4</sub> oxidation <sup>b</sup>	13 ( $\pm$ 1)	27 ( $\pm$ 4)	0.7/1.1
(3) [ $^3$ H]Spermidine + eIF-5A precursor <sup>c</sup>	42 ( $\pm$ 1)	92 ( $\pm$ 4)	2.1/3.8

<sup>a</sup>Incubation of the radiolabeled deoxyhypusine-containing eIF-5A in assay buffer and subsequent oxidation as described in legend to Fig. 1.

<sup>b</sup>Deoxyhypusine hydroxylase reaction and assay reactions were performed as described, except that NaIO<sub>4</sub> was omitted from the oxidation reaction.

<sup>c</sup>Incubation and subsequent oxidation of labeled spermidine plus unmodified eIF-5A (instead of radiolabeled deoxyhypusine-containing eIF-5A) as described above.

Table 2  
IC<sub>50</sub> values for enzyme inhibition and relative *K*<sub>d</sub> values for Fe(III) of different metal chelators

Ligand	IC <sub>50</sub> <sup>a</sup> (± σ) [nM]	Relative <i>K</i> <sub>d</sub> ± σ <sup>b</sup>
EDTA	279 ± 28.7	= 1
Ciclopiroxolamine	606 ± 33.1	> 1000
DPHP	752 ± 47.7	102 ± 39
Metipirox	2804 ± 239	1 ± 0.1
Mimosine	3268 ± 209	3 ± 0.5
α,α'-Dipyridyl	29,173 ± 192	< 3

<sup>a</sup>IC<sub>50</sub> values were calculated from data of the titration curves in Fig. 1.

<sup>b</sup>Relative *K*<sub>d</sub> values were determined by titration of their Fe(III) complexes with EDTA.

chromatographic separation of the amino acids, the essential steps used in all other deoxyhypusine hydroxylase assays [17,19,25]), can be avoided in this assay.

Treatment of CHO cells with different metal chelators, such as mimosine, kojic acid [14], α,α'-dipyridyl, desferal [11] and hydralazine [19], results in a potent inhibition of the deoxyhypusine hydroxylase activity in vivo. In extracts of metal chelator treated cells the content of fully modified hypusine was markedly reduced, while the amount of deoxyhypusine was increased. Furthermore, this inhibition of the deoxyhypusine hydroxylase correlates with a cell cycle arrest at the G1/S boundary [14,15]. Using the assay described above, the effect of the metal chelators EDTA, α,α'-dipyridyl, mimosine, ciclopiroxolamine (CPX), and its two derivatives metipirox and 4,6-diphenyl-1-hydroxy-pyridine-2-one (DPHP) was investigated in vitro (Table 2). As shown in Fig. 1, all metal chelators inhibit the deoxyhypusine hydroxylase in vitro with IC<sub>50</sub> values in the range of 0.3–29 μM. When the crystallized Fe(III)–CPX<sub>3</sub> complex was tested for enzyme inhibition, no effect on the

deoxyhypusine hydroxylase activity was observed (data not shown), which indicates that the inhibitory effect of the compounds tested depends on their metal ion chelating property. Therefore, the order of relative inhibitory potential of the chelators on the deoxyhypusine hydroxylase (expressed as IC<sub>50</sub> values) was compared with their metal ion chelating strength, as measured by their relative *K*<sub>d</sub> values for Fe(III). Table 2 shows a qualitative correlation between the decreasing IC<sub>50</sub> values and the increase in relative affinity of the chelators for Fe(III). The only exception to this is EDTA, which shows a weaker affinity for Fe(III), but is the most potent inhibitor of the deoxyhypusine hydroxylase in the series of chelators investigated here. As it is known from other metal ion dependent hydroxylases, the endogenous metal ion of these enzymes changes its ionization state during the catalytic cycle [20]. Since EDTA has a high affinity for divalent metal ions, it might exhibit its strong inhibitory effect by binding the endogenous metal ion of the deoxyhypusine hydroxylase in its divalent form.

Although these experiments prove an essential role of the

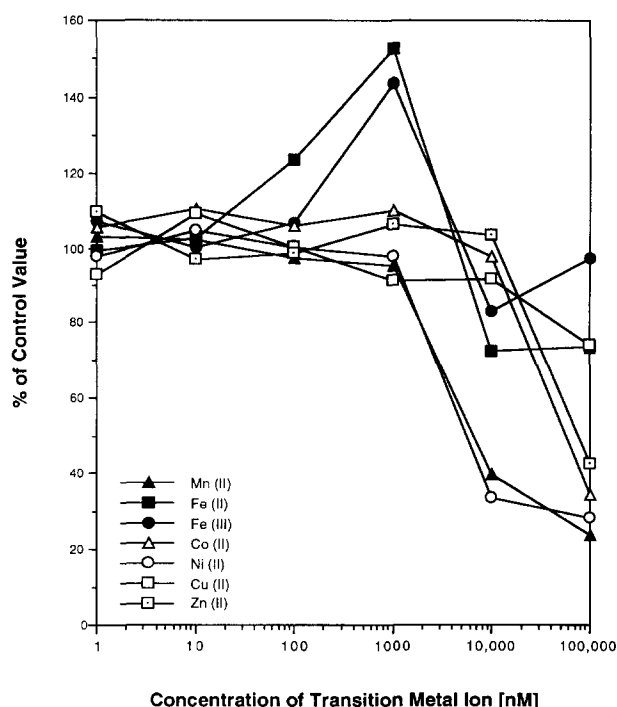


Fig. 4. Effect of transition metal ions on the deoxyhypusine hydroxylase activity. The indicated concentrations of the respective transition metal ions were added to the cell extracts prior to substrate incubation. The enzyme reaction and product determination were performed as outlined in the legend to Fig. 1. All values are given in percent of the control value, i.e. without addition of metal ion.

chelating capacity of these compounds in the inhibition of the deoxyhypusine hydroxylase, an additional specific interaction of the chelator compound with the enzyme itself cannot be excluded. To further investigate the mode of action of these chelators on the deoxyhypusine hydroxylase, reconstitution experiments using transition metal ions were performed. After inhibiting the deoxyhypusine hydroxylase with 3  $\mu$ M CPX, various amounts of Mn(II) acetate, Fe(II) sulfate, Fe(III) chloride, Co(II) acetate, Ni(II) acetate, Cu(II) chloride, or Zn(II) acetate were added prior to substrate incubation. The result of this experiment is shown in Fig. 2. Both, Fe(II) and Fe(III) are equally active in reconstituting the hydroxylase activity to the above control levels at a concentration of 1  $\mu$ M. At higher concentrations, the deoxyhypusine hydroxylase activity is again inhibited, presumably due to unspecific binding of the ferric ions to the enzyme. Partial reconstitution of the enzyme activity was also observed with Cu(II) and – to a lesser extent – with Zn(II). Addition of Co(II) resulted only in a marginal reconstitution, whereas Mn(II) and Ni(II) further inhibited the deoxyhypusine hydroxylase. Since a full recovery of the deoxyhypusine hydroxylase was achieved by addition of either Fe(II) or Fe(III) the formation of a stable complex of the deoxyhypusine hydroxylase, the metal ion and CPX, based on specific binding of the chelator to the enzyme, can be excluded. Rather, CPX seems to form a metal ion complex, which rapidly dissociates from the enzyme. Therefore, the mode of action of CPX is due to metal ion chelation.

The various transition metal ions differ in their capacities to reconstitute the deoxyhypusine hydroxylase activity. This might, on the one hand, indicate the specificity of the de-

oxyhypusine hydroxylase for ferric ions. On the other hand, this result might also reflect differences in the relative affinities of CPX for these metal ions. In the latter case, binding of the respective metal ion to CPX might lead to the equivalent displacement of the endogenous metal ion of the deoxyhypusine hydroxylase from CPX, which then restores enzyme activity accordingly. In order to investigate this issue, a metal ion reconstitution experiment was performed, using EDTA instead of CPX for inhibiting the deoxyhypusine hydroxylase (Fig. 3). Again, ferric ions were capable of fully restoring the enzyme activity at a concentration of 1  $\mu$ M. Also, addition of Zn(II) partially restored the deoxyhypusine hydroxylase, even though higher concentrations are necessary. Interestingly, also Mn(II) addition resulted in a partial activation of the enzyme, which was not observed after inhibition with CPX. Co(II), Ni(II) and Cu(II) did not show any activating effect. By comparing the results of both reconstitution experiments (Figs. 2 and 3), we conclude that only ferric ions show a qualitatively and quantitatively consistent effect in fully restoring the deoxyhypusine hydroxylase activity. The effect of the other transition metal ions on the activity of the deoxyhypusine hydroxylase strongly depended on the metal chelator used and therefore, seem to reflect the chelators' affinity for the respective metal ion.

Finally, the effect of transition metal ion on the deoxyhypusine hydroxylase activity without prior inhibition by metal chelators was investigated. With the exception of Fe(II) and Fe(III), none of the metal ions showed any activating effect, but inhibited the enzymatic activity at concentrations of > 1  $\mu$ M (Mn(II), Ni(II)) or > 10  $\mu$ M (Co(II), Cu(II), Zn(II)), respectively (Fig. 4). In contrast, both Fe(II) and Fe(III) stimulated the activity of the deoxyhypusine hydroxylase by 1.5-fold at a concentration of 1  $\mu$ M. Again, at higher concentrations also ferric ions showed an inhibitory effect. Experiments showing the partial inhibition of the deoxyhypusine hydroxylase by transition metal ions have been previously published. However, the stimulating effect of ferric ions at low concentrations was not described, which might, in part, reflect its state of iron saturation [17].

Ferric ions were the only transition metal ions, which at a concentration of 1  $\mu$ M stimulated the endogenous deoxyhypusine hydroxylase activity and reproducibly restored its activity, both after CPX and EDTA inhibition. They, therefore, appear to be endogenous constituents of the deoxyhypusine hydroxylase. A distinction between the relative effect of Fe(II) and Fe(III) was not possible, since ferric ions are only stable in their reduced form under the assay conditions used. Furthermore, the deoxyhypusine hydroxylase catalyzes a redox reaction and the essential endogenous metal ion also may well change its ionization state during the catalytic cycle.

In agreement with several other reports [11–14,17,19] we found that EDTA, mimosine,  $\alpha,\alpha$ -dipyridyl, CPX and its derivatives metipirox and DPHP potently inhibit the deoxyhypusine hydroxylase. The qualitative correlation of the  $IC_{50}$  values for enzyme inhibition with the metal ion chelating properties at similar conditions, together with the fact that the enzyme activity can be fully restored by addition of equimolar amounts of ferric ions, led us to the conclusion that this inhibition is caused by metal ion chelation. A specific inhibition of the deoxyhypusine hydroxylase [16] due to direct binding of the inhibitory compound could be excluded both for CPX and for EDTA. Strong metal ion chelators, like mimosine and CPX were re-

ported to influence a variety of cellular processes [26–29]. The correlation of the *in vivo* inhibition of the deoxyhypusine hydroxylase, the arrest of the cell cycle at the G1/S boundary [14], and the non assembly of certain mRNAs into polysomes observed after mimosine treatment [15] should, therefore, be cautiously interpreted.

With the rapid and specific assay for the deoxyhypusine hydroxylase presented, the enzymatic mechanism of the deoxyhypusine hydroxylase and its inhibition by polyamines [13] can further be investigated. Moreover, this assay technique considerably facilitates the establishment of a purification protocol for this enzyme.

**Acknowledgements:** We gratefully acknowledge Dr. W. Phares for continuous support during the experiments, and Dr. H. João for stimulating discussions and helpful suggestions. We are indebted to Dr. F. Lottspeich for helpful discussion and critically reading of the manuscript and thank Dr. J. Hauber for his interest in this project.

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