Detergent-mediated destaining of Coomassie Brilliant Blue-stained SDS polyacrylamide gels

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A simple and one-step detergent-mediated destaining procedure for SDS Polyacrylamide gels for proteins is described. Suspension (5%, w/v) of a commercially available household detergent, Vim Ultra, has been found to be very efficient in destaining polyacrylamide gels without interfering with the resolution of proteins. As compared to the routinely used solvent (methanol-acetic acid-water)-mediated destaining procedure, the present method is economical and user-friendly.

Polyacrylamide gel electrophoresis for analysis and characterisation of proteins is a routine procedure employed by researchers in the field of protein biochemistry. From the beginning of development of this valuable method, efforts are being made to improve on its quality in various directions and the enthusiasm still continues. Among many such parameters, visualisation of proteins following electrophoretic separation has been given enough attention. Visualisation of proteins in polyacrylamide gels requires staining of the proteins and destaining of the gels to get a clear background. For this purpose, various staining techniques have been used. However, the most popular techniques are Coomassie Brilliant Blue (CBB) and silver staining due to their proven reliability, simplicity and economy. The standard CBB staining procedure involves immersing the gel in a solution of methanol / acetic acid / water containing 0.1% (w/v) CBB followed by destaining with the same solution excluding the stain. Traditionally, staining and destaining usually require 3-6 and 10-48 hr, respectively. Though destaining can be shortened by use of absorbents (e.g., charcoal containing sponges), such procedures require more apparatus and / or chemicals and are not simple. At the same time these solvents / chemicals are also toxic for handling on routine basis. In the present communication, a simple and single-step destaining procedure using a commercially available household detergent, Vim Ultra has been described. This procedure is easy to use and the detergent is inexpensive and less toxic as compared to the solvents that are used in the conventional destaining procedures.

Protein sample used in these experiments was extracted from chickpea in lysis buffer containing the following: 20 mM Tris-HCl, pH 8.0; 1 mM EDTA; 1 mM PMSF; 0.1% Triton X-100. Extract was centrifuged at 15,000 rpm for 20 min and the soluble supernatant was used for analysis. Proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) according to Laemmli. Immediately after electrophoresis gels were stained overnight in methanol / acetic acid / water (5:1:4) containing 0.125% (w/v) CBB R-250. For destaining, various concentrations of different detergents, namely, Vim Ultra and Surf Excel (Hindustan Lever Ltd., Mumbai) and Ariel (Procter & Gamble Home Products Ltd., Mumbai) were used, either alone or as a mixture of two (1:1). The detergent suspensions (5%) were filtered through Whatman No. 1 filter paper to eliminate large particles, before their use. For each experiment, one gel was destained by the routine procedure (as control) using methanol / acetic acid / water (4:1:5) along with gels destained by detergents. Optimum results were obtained with 5% suspension of Vim Ultra. After partial destaining for about 3 hr, the gels were transferred to 7.5% acetic acid overnight for obtaining a clear background.

The resolution of protein bands, in general, in terms of numbers and intensities on the gel destained by the detergent Vim Ultra (Fig. 1B) was very similar to that destained by the routine procedure (Fig. 1A). The background in both the gels was indistinguishable. The detergent-mediated destaining took almost an hour more as compared to that taken
Table 1—Summary of results on destaining with various detergents

<table>
<thead>
<tr>
<th>Destaining method used</th>
<th>Time taken for partial destaining* (hr)</th>
<th>Results obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Traditional solvent-mediated (as control)</td>
<td>2</td>
<td>Clean background, good resolution</td>
</tr>
<tr>
<td>2. Detergent-mediated a) Vim Ultra (5% w/v)</td>
<td>3</td>
<td>Clean background, resolution similar to control</td>
</tr>
<tr>
<td>(b) Aerial (5% w/v)</td>
<td>3</td>
<td>Loss of resolution of less intense bands, colour of protein bands changed from blue to yellowish green</td>
</tr>
<tr>
<td>(c) Surf Excel (5% w/v)</td>
<td>3</td>
<td>Loss of protein bands of low intensity</td>
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* For complete destaining, gels were kept in 7.5% acetic acid overnight.

by the routine destaining procedure. However, the time taken could be shortened if the destaining is done with a warm (40°C) suspension of the detergent. When the cost of destaining per gel (mini gel) was calculated, detergent-mediated destaining was found to be about 6-times cheaper as compared to the routine destaining procedure (Rs. 2/- vs Rs. 12/-). These results, therefore, suggest that this new procedure of detergent-mediated destaining of polyacrylamide gels could be used as an alternative to the routine destaining procedure.

Two other household detergents, namely, Surf Excel and Aerial, either alone or as a mixture (1:1) of two in various combinations were also used (data not shown). The protein profile of the gel destained with Vim Ultra was the best as compared to those destained by the other two detergents, using either alone or as mixtures, in terms of resolution and status of the gel. In general, the other two detergents resulted in loss of stain in some minor protein bands with molecular weight of wide range all over the gel. Furthermore, Aerial caused a change of colour of the protein bands from blue to yellowish green, which was found unsuitable for documentation (photographic) purpose. The summary of results obtained with all these detergents is presented in Table 1.

Household detergents are cheap and easily available as compared to organic solvents present in routine destainer. Beside this, the detergents at the concentration used are much less toxic as compared to methanol and acetic acid used in traditional destainer, so the use of the detergents as destainer is much more eco-friendly. In conclusion, Vim Ultra, a household detergent, could be used as a suitable alternative reagent for destaining polyacrylamide gels in laboratories engaged in protein research, and more so in schools and colleges where this technique is used for demonstration purpose.

Fig. 1—Destaining of SDS polyacrylamide (10%) gels by routine solvent-mediated destaining (A) and detergent-mediated destaining (B) procedures. Lane1, Molecular weight marker proteins; lanes 2-9, same quantity (35 μg) of chickpea extract. Electrophoresis was carried out at 25 mA (constant current), gels were stained in 0.125% CBB overnight and destained by either Methanol: Acetic acid: H2O (40:10:50) (Panel A) or by 5% Vim Ultra (Panel B) for 3hr and then transferred to 7.5% Acetic acid.

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References
Lead Exposure and Heat Shock Inhibit Cell Proliferation in Human HeLa and K562 Cells by Inducing Expression and Activity of the Heme-regulated eIF-2α Kinase

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We have used human cell lines, namely, K562 and HeLa cells as model systems in understanding the mechanism of lead toxicity and heat shock, that may be mediated by the heme-regulated eIF-2α kinase which is also called the heme-regulated inhibitor (HRI). RT-PCR analysis using HRI-specific primers indicated a two- to three-fold increase in HRI expression in K562 and HeLa cells exposed to lead acetate and heat shock, respectively. Further, in vitro eIF-2α kinase assay indicated a two- to three-fold increase in HRI kinase activity during lead toxicity in K562 cells. This increase in HRI expression and its activity was accompanied by a significant decrease in cell proliferation and cell viability. This is therefore, the first report indicating that both heavy metal exposure and heat shock cause inhibition of protein synthesis not by activation of HRI alone but by its over-expression as well as activation. Our data indicate further that lead-induced inhibition of cell proliferation may be caused due to inhibition of protein synthesis resulted due to induced expression and activity of HRI.

Keywords: Heme-regulated inhibitor; mRNA expression; Protein kinase activity; Heat shock; Heavy metal exposure; Protein synthesis

INTRODUCTION

The role of the heme-regulated eukaryotic initiation factor 2α (eIF-2α) kinase in regulating initiation of protein synthesis in reticulocytes and a number of non-erythroid cells is well established [1–4]. Upon activation, the heme-regulated eIF-2α kinase, a Ser/Thr protein kinase, inhibits protein synthesis, and is therefore also called the heme-regulated inhibitor (HRI). The activation of HRI caused due to heme deficiency or a variety of other conditions, such as heat shock, heavy metal toxicity, treatment with N-ethylmaleimide (NEM) and oxidized glutathione (GSSG), leads to phosphorylation of its substrate, the 38 kDa α subunit of eIF-2. eIF-2 being rate limiting needs to be recycled during initiation of protein synthesis. However, phosphorylated eIF-2 (eIF-2αP) being unable to recycle results in the inhibition of protein synthesis [5–8].

Among the various conditions and reagents that activate HRI, and inhibit protein synthesis, heavy metal exposure and heat shock are of interest here. Heavy metals, such as zinc, copper, cadmium, mercury, and lead are minor yet ubiquitous components of the biosphere. Some of these, zinc and copper, in particular, which participate in a variety of enzymatic reactions, are essential trace elements for all life forms but are toxic when present in inappropriately high concentrations [9]. The release of these heavy metals due to industrial activities causes their accumulation in high quantities, thereby resulting heavy metal poisoning in living organisms. In humans, chronic exposure of lead results anemia [10,11]. Therefore, it is crucial to understand the molecular mechanism of heavy metal poisoning.

Many agents which commonly elicit stress response in eukaryotic cells are also capable of generating reactive oxygen species, reacting with reduced sulfhydryl groups, or inhibiting the ability of the cell to generate reducing equivalents. Among these agents, the heavy metal ions, AsO₃²⁻, Cd²⁺, Cu²⁺, Hg²⁺, Pb²⁺ and Zn²⁺ in particular, have been shown to inhibit protein synthesis and disaggregate polyribosomes, while these elicit stress response in a variety of eukaryotic cell types and tissues [12–14].

Although there are some data available from in vitro studies that heavy metals induce HRI activity and thus
inhibit protein synthesis [14], not much is known on the
effect of heavy metals in organisms or intact cells in vitro.
In this investigation, therefore, we have addressed these
questions by designing experiments using human cell lines
as models. We have used human K562 and HeLa cells for
lead exposure as well as heat shock. Our results indicate
that under both the conditions, there is a significant
increase in the expression as well as eIF-2α kinase activity
of HRI. Thus, our results, for the first time, suggest that a
combination of higher level of expression and activity of
HRI may be instrumental in the inhibition of protein
synthesis during these stresses.

MATERIALS AND METHODS

All the general laboratory chemicals were purchased from
either Sigma Chemical Co., USA or Life Technologies
(GIBCO BRL), USA. HeLa and K562 cells were obtained
from National Centre for Cell Sciences, Pune, India. First
strand cDNA synthesis kit for RT-PCR was purchased from
Roche Molecular Biochemicals, Germany. Antibodies
were purchased from Sigma Chemical Co. Radioisotope
(γ-32P) ATP was purchased from Board of Radiation and
Isotope Technology (BRIT), India.

In vitro Culture of K562 and HeLa Cells

HeLa and K562 cells were cultured in vitro and
maintained in DMEM supplemented with 10% FBS
(37°C with 5% CO2), by subpassaging them on every fifth
day. For all experiments, cells were cultured with a
seeding density of 5 × 10^3 cells/ml.

Exposure to Lead Acetate and Heat Shock

K562 cells were exposed to different concentration of lead
acetate (10, 100 and 500 μg/ml) after 24 h of culture. For
heat shock, both K562 and HeLa cells cultured at 37°C
were transiently exposed to 42.5°C for 1 h. Cell growth
and proliferation were measured by determining cell
numbers prior to and after manipulations.

RNA Extraction and RT-PCR Analysis

Total RNA was extracted from the control and
experimental cell samples as per the manufacturer’s
(Life Technologies, USA) protocol using TRIZOL®
reagent. First strand cDNA was synthesised from RNA
samples (1 μg) using the cDNA synthesis kit for RT-PCR
(AMV) from Roche Molecular Biochemicals, Germany,
as per specifications provided in the kit. PCR amplification
of HRI cDNA was carried out using HRI-specific primers
(1 μM each) as described earlier [15]. The two primers
that were used for PCR are as follows: 5’-AAAAATAGGG-
AGACTTTTGCTGGCCTGCGGCCACATC-3’ and
5’-CTCCATATCGTGGCCAGGCTTGAAGA-3’.
The PCR was carried out for 40 cycles (97°C for 2 min, 94°C
for 1 min, 47°C for 2 min, 70°C for 1 min). Simultaneous
amplification of human β-actin sequence was done
using actin-specific primers: 5’-GTGGGGCGCCCCAG-
GCACCA-3’ and 5’-CTCCTTAAATGTACCGACGA-
TTTC-3’, as an internal control. PCR products were
analysed on a 1.2% Agarose gel followed by ethidium
bromide staining. The results were analysed using a gel
documentation system (UVP, UK).

Protein Extraction and Estimation of Protein
Content by Bradford’s Method

Proteins were extracted from K562 cells, using lysis buffer
(50 mM Tris–HCl pH 8.0; 5 mM EDTA; 0.1% Triton X-
100; 1 mM PMSF). Protein content of the cell extracts was
determined by Bradford’s micro estimation method [16].

In vitro eIF-2α Kinase Assay

Protein kinase assay mixture containing 5–8 μCi of
[γ-32P] ATP was added to the protein extracts of control
as well as lead acetate treated (100 and 500 μg/ml) K562
cells, and the kinase assay was carried out at 30°C for
20 min, in presence of exogenously added rabbit eIF-2 as
the substrate, as described earlier [17]. Assays were
carried out under three different conditions, namely,
without heme, with the addition of hemin chloride
(20 μM) and N-ethylmaleimide (NEM). Reaction was
stopped by the addition of NaF-EDTA, and proteins were
precipitated at pH 5.0 by addition of 0.5 M acetic acid.
Protein pellet obtained by centrifugation at 10,000 g
was treated with sample buffer, boiled for 3–5 min and
analysed by SDS PAGE (10%). Gel was stained with
0.25% Coomassie Brilliant Blue R-250, destained, dried
and exposed to X-Ray film for 4–5 days at room
temperature.

SDS PAGE and Western Blot Analysis

Samples containing equal quantity of proteins were
denatured in sample buffer for 3–5 min at 100°C and
analysed by SDS PAGE [18]. Electrophoresis was
carried out at a constant current of 25 mA at room
temperature. Following SDS PAGE, proteins were
electrophoretically transferred to a nitrocellulose mem-
brane [19]. Blots were then processed for immuno-
reactivity using anti-hsp90, anti-hsp70 and anti-p34cdc2
monoclonal antibodies. In brief, blots were saturated
with 3% BSA for 4 h, and incubated overnight with
primary antibody in phosphate buffered saline (PBS,
PH 7.4) and then with alkaline phosphatase-conjugated
secondary antibody for 4 h at room temperature.
Following each antibody incubation, blots were washed
three (15 min each) in PBS. Blots were developed for
colour reaction using NBT-BCIP, as the substrate. The
results were analysed using UVP-gel documentation
system (Ultra violet products, UK).
RESULTS

Effect of Lead on K562 Cell Proliferation

In order to determine the optimum concentration of lead acetate required for affecting the cell proliferation in vitro, a series of pilot experiments using different concentrations of lead acetate ranging from 2-500 μg/ml were carried out (data not shown). Results obtained from these experiments indicated that a minimum of 10 μg/ml lead acetate is required to affect cell proliferation. Therefore, in subsequent experiments, three different concentrations of lead acetate i.e. 10, 100 and 500 μg/ml were used. In all these concentrations, lead acetate showed an inhibitory effect on cell proliferation and viability after 24 h of exposure, with highest inhibition at 500 μg/ml concentration (Fig. 1). After 48 h, cells under 10 and 100 μg/ml of lead exposure appeared to recover. However, no significant recovery was seen in 500 μg/ml concentration, indicating that the cells were not able to recover from toxicity at such high concentration (Fig. 1).

The specificity of lead effect was further ascertained by exposing the cells to acetates of magnesium and sodium in addition to that of lead (500 μg/ml). Results indicated that lead caused a significant decrease (about 3-fold) in cell population as compared to that in the control sample within 24 h of exposure, with a further decrease at 48 h. Contrary to this, the other acetates did not have any effect on cell proliferation (data not shown), indicating the specificity of lead-effect.

Effect of Lead Acetate on HRI Expression in K562 Cells: RT-PCR Analysis

In order to determine the effect of lead acetate on HRI expression, K562 cells were exposed to various concentrations of lead acetate (10, 100 and 500 μg/ml) for 24 h. Total RNA was extracted and used for RT-PCR experiments. RT-PCR analysis using HRI-specific primers revealed an increased expression of HRI in lead-exposed cells (Fig. 2). As seen in Fig. 2a, the 230bp HRI amplificate was 2–3 times more in the 100μg/ml lead acetate-treated cells as compared to that in the control sample. Human β-actin, which was used as an internal control, did not show any change (600bp amplificate). Further, in a time-course experiment (2–24 h), the effect of lead acetate on HRI expression appeared to be highest at 8 h exposure (Fig. 2b).

Effect of Heat Shock on HRI Expression in HeLa and K562 Cells: RT-PCR Analysis

Heat shock, another type of stress, is known to activate HRI and inhibit protein synthesis. Therefore, it was of interest to determine the expression of HRI during heat shock. For this experiment, in addition to K562 cells, we have also used HeLa cells, in which the heat shock response has been well established. RT-PCR analysis indicated a two-fold increase in HRI expression in HeLa cells exposed to heat shock (Fig. 3). On the other hand, HRI expression in K562 cells did not increase significantly during heat shock (Fig. 3). In the same samples, there was no change in actin expression (a 600 bp amplificate), during the transient heat shock of 1 h duration. Further, it is to be noted here that under normal condition, the basal level of HRI expression appeared to be more in K562 than in HeLa cells.

Effect of Lead Exposure on HRI Kinase Activity in K562 Cells

In order to verify if the increase in HRI expression during lead acetate-treatment of K562 cells is also accompanied by an increase in eIF-2α kinase activity, HRI kinase assay of all the three samples (control, 100 and 500 μg/ml lead acetate-treated samples) were carried out as described in the "Materials and Methods" section. As seen in the autoradiogram (Fig. 4a), eIF-2α phosphorylation is almost undetectable in the control sample (lanes 1–3), whereas it

![Image](https://example.com/image.png)  
**FIGURE 1** Effect of lead acetate on K562 cells. K562 cells (5 × 10⁷ cells/ml) were grown in DMEM with 10% FBS for 24 h and were then treated with various concentrations (10, 100 and 500 μg/ml) of lead acetate. Cell counts were taken at every 24 h for 2 days, and the data were analysed. The data of four individual experiments are summarised in this figure.
increased significantly in samples treated with lead acetate of both the concentrations (Fig. 4, lanes 4–9). In each set of three lanes, the first, second and third assay samples were in absence of hemin (−h), in presence of hemin (+h) and in presence of NEM (+NEM), respectively. The hemeregulated eIF-2α kinase activity, as indicated by its suppression in presence of hemin, appeared to be concentration-dependent, higher in 500 μg/ml (lanes 7–9) as compared to that in 100 μg/ml of lead acetate (lanes 4–6). Interestingly, the hemin-sensitivity decreased in lead acetate-treated samples as a function of concentration. From the densitometric scan (Fig. 4b), it appeared that the kinase activity increased to almost 4-fold during 500 μg/ml lead exposure as compared to that in the control.

**FIGURE 2** Effect of lead acetate on HRI expression in K562 cells. Cells were exposed to either different concentrations of lead acetate (a), or to 100 μg/ml lead acetate for various time periods (b). Total RNA was extracted and RT-PCR was carried out using 1 μg of total RNA. PCR products (HRI- and actin-specific) were analysed by 1.2% Agarose gel electrophoresis. (a) Lane 1, control; lanes 2, 3 and 4, 10, 100 and 500 μg/ml lead acetate-treated samples, respectively; M, DNA size marker (100 bp ladder). (b) Lanes 1, 2 and 3, 2, 8 and 24 h of lead exposure. (a') and (b'), quantification profiles of HRI amplify (230 bp) in gel (a) and (b), respectively.

**FIGURE 3** Expression of HRI in HeLa and K562 cells subjected to heat shock. (a) Cells were subjected to heat shock at 42.5°C for 1 h, total RNA was extracted and RT-PCR was carried out. The amplified products (HRI and actin) were analysed by Agarose gel electrophoresis (a). M, DNA size marker (100 bp ladder); lanes 1 and 2, HeLa and lanes 3 and 4, K562; lanes 1 and 3, control and 2 and 4, heat shocked samples. (b), quantification profile of HRI amplify (230 bp) in gel (a).

proteins namely, hsp90 and hsp70, and a cell cycle regulatory protein, p34cdc2. The results obtained from Western blot experiments indicated a rather marginal increase in the quantity of hsp90 (Fig. 5a) and hsp70 (Fig. 5b) in the 500 μg/ml lead acetate-treated sample as compared to the control, whereas it remained unchanged in the 100 μg/ml lead acetate-treated samples. On the other hand, there was a significant increase in the level of p34cdc2 protein during lead exposure as compared to the control (Fig. 5c). Further, this protein also showed a lower mobility at 500 μg/ml lead concentration (Fig. 5c).

**DISCUSSION**

In this paper, we demonstrate for the first time that in *in vitro* cultured human K562 cells, lead exposure causes not only activation of HRI but its overexpression as well. Although heavy metals, namely, cadmium, mercury and lead have been shown to inhibit protein synthesis by stimulating eIF-2α phosphorylation through activation of HRI in a cell-free reticulocyte lysate [14], nothing was known on the effect of heavy metals on HRI expression and protein synthesis *in vivo*. Our results also indicate that the level of HRI expression and its activity were lead concentration-dependent. Interestingly, results of the time-course experiment of lead exposure indicated that a period of 8 h exposure induced the highest level of expression of HRI. On the other hand, during heat shock, an increased expression of HRI took place within an hour. Similarly, De Benedetti and Baglioni [20] have reported an increased HRI activity and inhibition of protein synthesis in HeLa cells within 30 min–1 h of exposure. These results thus, suggest that lead exposure, although a type of stress, is different from other cytoplasmic stresses, with reference to the stress response.

Heat shock response of HeLa cells and inhibition of general protein synthesis through activation of HRI is well-established [20]. Here we have determined the expression
of HRI after 1 h of heat shock at 42°C in both HeLa and K562 cells. Although the level of HRI expression increased in HeLa cells to 3-fold, a much less increase was observed in K562 cells. There could be two possible explanations for this. (1) The basal level of HRI expression is higher in K562 cells, therefore, it does not increase much during heat shock. Indeed, it has been shown that erythroid cells express HRI almost 10 times more than other cell types [21]. (2) These two cells may be different in heat shock response with reference to both temperature and duration. Even in HeLa cells, differential HRI activation has been observed at two different temperature, 42°C and 44°C [22]. However, further studies will be required to verify the latter possibility.

HRI is known to interact with Hsps, Hsp90 and Hsp70 in particular, during stress [23,24]. Although we have not determined their interaction with HRI per se during lead exposure of K562 cells, no significant change in their quantity was detected by Western blot analysis (Fig. 5a,b). However, at the organism level in rabbit, there was significant increase in the quantity of both Hsp70 and Hsp90 during lead exposure leading to anemia [25]. This difference is probably due to the fact that in short exposure, the cells are able to recover and therefore the level of Hsps does not show any significant change. The present study, therefore, strengthens further that HRI can suitably be used as a more appropriate indicator of lead exposure of both short and long duration especially as compared to the Hsps.

We have determined the quantitative change of a cell cycle regulatory protein p34cdc2 by Western blot using anti-p34cdc2 antibody. Since cell proliferation was affected due to lead exposure, it was of interest to determine changes of this protein that might indicate if cell cycle regulation is altered. In addition to an increased level of p34cdc2 at 500 μg/ml of lead concentration, this protein had a lower mobility (Fig. 5c). This might be indicative of a change in the phosphorylation status of this protein, which is known to have a bearing on the regulation of cell division.
We have also attempted to determine if lead-induced decline in cell proliferation is caused due to apoptosis. We have detected a rather low level of apoptosis (8%) that might be contributing to the depletion of cell population at 500 μg/ml of lead concentration (data not shown). However, at an organism level, it appears that apoptosis takes place in a significant way as an effect of lead exposure, particularly in the germ cells in rat testis [26].

Considering the results, we conclude that (1) these cell lines can be very usefully utilised as models to determine the molecular mechanism of lead toxicity, despite the differences in the response between cells and the organisms, (2) expression of HR1 may be considered as an indicator of lead toxicity, at both the levels of cells as well as organisms and (3) induced expression of HR1 during lead exposure is important in combating the stress, as in other types of cytoplasmic stresses [27], by inhibiting global protein synthesis in cells.

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