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Quantitative and qualitative analysis of DNA extracted from postmortem muscle tissues

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Summary. DNA extracted from 33 postmortem muscle specimens was analyzed using MZ 1.3, a hypervariable minisatellite probe, as well as locus-specific minisatellite probes (g3, MS1 and MS43). After storage at −25°C for 10 months, DNA from all the samples was partially (approximately 21% of total DNA) degraded even when autopsy was performed 1 day post mortem. However, more than 90% of DNA samples up to at least 3 days post mortem were suitable to obtain good restriction fragment length polymorphism (RFLP) patterns. When small strips of specimen were stored for 8 days at room temperature in moist chambers, approximately 42% of total DNA was degraded. Only 30% of these DNA samples still showed good RFLP patterns. However, no obvious relation between qualities of DNA analyzed by detection of RFLP and quantities of total and high-MW DNA became apparent. A case of familial relationship was ascertained by DNA fingerprints. Since DNA of good quality can be recovered from muscle tissues in large quantities, DNA extraction from muscle tissues and detection of RFLP patterns should be very useful for individual identification in autopsy cases.

Key words: DNA fingerprinting – Locus-specific probes – DNA stability – Muscle tissues – Individual identification

Zusammenfassung. Genomische DNA wurde aus 33 Muskelgewebsproben postmortal extrahiert und im Southern Blot-Verfahren sowohl mit der Multilocus-Minisatelliten-DNA-Sonde MZ 1.3 als auch mit Locus-spezifischen DNA-Sonden (g3, MS1 und MS43) analysiert. Nach Lagerung der Gewebsproben bei −25°C für 10 Monate war die DNA von allen Proben partiell de-

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gradiert (ca. 25% Anteil degradierter DNA an Gesamt-DNA), auch wenn die Obduktion innerhalb eines Tages nach Eintritt des Todes erfolgte. Dennoch waren über 90% der DNA-Proben, die bis zu drei Tage nach Tod entnommen worden waren, für eine Southern Blot-Analyse geeignet. Bei Lagerversuchen von kleinen Muskelgewebsproben bei Raumtemperatur in einer feuchten Kammer für acht Tage waren ca. 42% der Gesamt-DNA degradiert. Nur von 30% dieser gelagerten DNA-Proben waren noch Minisatelliten-DNA-Fragmente darzustellen. Es war jedoch keine deutliche Abhängigkeit zwischen dem jeweiligen Anteil von hochmolekularer DNA zu Gesamt-DNA einer Probe und der Detektion von Minisatelliten-DNA-Fragmenten zu erkennen. Zusätzlich konnte in einem Fall eine Verwandtschaftsbeziehung anhand der Minisatelliten-DNA-Fragmente mit MZ 1.3 untersucht werden. Da insgesamt genomische DNA in guter Qualität und ausreichender Menge aus Muskelgewebsproben isoliert werden konnte, erscheint diese Methode zur Darstellung von individuellen DNA-Fragmentmustern bei forensischen Fragestellungen in Autopsiefällen sehr nützlich.

Schlüsselwörter: Genetischer Fingerabdruck – Locus-spezifische DNA-Sonden – DNA-Stabilität – Muskelgewebe – Identifizierung

Introduction

Hypervariable minisatellite DNA probes detect individual-specific fragment patterns in genomic DNA of most higher organisms [1, 2]. They have been used to analyze a wide variety of problems in the field of forensic science [3, 4]. Recently, a new minisatellite probe, MZ 1.3, has been isolated, and this probe has been shown to be very useful for DNA fingerprints with radioactive and nonradioactive detection systems [5]. In addition, so-called single-locus probes have also been devised and found to be good tools to detect locus-specific minisatellites [6, 7].

It is known that DNA from cadaverous muscle specimens is more stable than DNA from cadaverous blood specimens [8]. Recently, we were able to obtain autopsy material from victims that had died immediately and simultaneously from an accident. So we collected small muscle samples from each cadaver and examined the stability of DNA from those samples. The DNA from postmortem muscles was analyzed qualitatively and quantitatively by spectrophotometry as well as by using both the MZ 1.3 probe and the single-locus probes g3, MS1 and MS43 [6].

Materials and methods

Muscle specimens. Specimens of psoas muscle were collected from 33 accident victims subjected to autopsy (23 males and 10 females) in the Institute of Legal Medicine, Mainz University. All of the victims were visiting a "Flight Show" and died immediately and almost simultaneously, as they were hit by burning debris from the collision of two airplanes in the sky. The median age of the victims was 35.1 (range 12–68) years. The cadavers were kept at 4°C. 25 cases were autopsied on the following day, 7 cases 2 days and 1 case 3 days after the incident.

Autopsies revealed that the causes of death were severe injuries and/or burns. The specimens were frozen at −25°C as soon as possible and stored for 10 months. For further examinations, small pieces (1–2 g) of muscle tissue were kept for 8 days at room temperature (20°C) in moist chambers at 95%–100% relative humidity.

Extraction of DNA. Tissue samples were mechanically homogenized in 10 ml of a buffer containing 10 m*M* Tris, 75 m*M* NaCl and 25 m*M* EDTA (pH 7.5). After homogenization, Proteinase K (final concentration: 0.7 mg/ml, Boehringer, Mannheim, FRG) and sodium dodecylsulfate (SDS; final concentration: 0.5%) were added. The solutions were incubated overnight at 37°C. The proteins were extracted from DNA once with phenol, twice with phenol/chloroform/isoamylalcohol (25:24:1), and finally once with chloroform/isoamylalcohol (24:1). DNA was precipitated by adding 1/10 vol. of 3 *M* sodium acetate (pH 5.2) and 2 vol. of absolute ethanol. After drying, the DNA was resuspended in 1 ml 10 m*M* Tris, 1 m*M* EDTA (pH 7.4). To remove contaminating RNA, RNase treatment (final concentration: 0.1 mg/ml) was performed. The DNA was stored at 4°C. The DNA yield was determined with a spectrophotometer by reading the absorbance at 260 nm. An optical density (OD 260) of 1.0 was calculated as corresponding to 50 µg/ml DNA.

Estimation of high-molecular-weight DNA. Twenty microliters of the DNA solution, which had been obtained by extracting DNA from 26 muscle samples before and after storage for 8 days at room temperature, were loaded on a 0.5% agarose gel in 89 m*M* Tris, 89 m*M* boric acid and 0.8 m*M* EDTA (TBE) (pH 7.7) including 0.5 µg/ml ethidium bromide. The gel was electrophoresed at 40 V for 3 h. The gel was cut into two pieces, one of which included high-molecular-weight (MW) DNA (> 15 kb) and the other low-MW DNA (< 15 kb) using *Xho*I-digested lambda DNA as a length marker. The DNA from the gel slices was electroeluted into dialysis tubing in TBE at 250 V for 3 h. When all DNA was removed from the gel slices, the dialysis solution was transferred into glass tubes that had been pretreated with Repel-Silane (Pharmacia-LKB, Freiburg, FRG). The solutions were extracted with butanol to reduce the aqueous phase, and distilled water was added to a final volume of 1 ml. The DNA yields were determined using a spectrophotometer at 260 nm absorbance. The ratio (high-MW DNA yields/total [high- + low-MW] DNA yields) was calculated. For statistical analysis, the paired or unpaired *t*-test was used.

Southern blot analysis. Ten micrograms of high-MW DNA were digested with 50 U of *Hin*fI and incubated at 37°C overnight. The completeness of digestion was examined using small test gels. When the digestion was incomplete, the samples were either ethanol precipitated and the digestion was repeated or 50 U of *Hin*fI were added and an additional one-day incubation was done. Restriction fragments were separated in a 0.7% agarose gel (20 × 25 cm) in TBE at constant voltage (40 V) for 30 h. After electrophoresis and depurination with 0.25 *M* HCl for 15 min, Southern blotting was performed in 0.4 *N* NaOH [9]. The transfer of DNA onto the nylon membrane (NY13, Schleicher & Schüll, Dassel, FRG) was completed after 5 h. The membrane was washed in neutralization buffer (1.5 *M* NaCl, 0.5 *M* Tris, pH 8) for 5 min and then in 5 × SC (1 × SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate, pH 7) for 5 min. The dried membrane was baked for 2 h at 80°C.

Radioactive detection. MZ 1.3, a multi-locus minisatellite probe [5], was labelled by nick translation [10] to a specific activity of 1×10^8 cpm/µg using a commercial kit (BRL, Eggenstein, FRG) and alpha-^{32}P-dATP (3000 Ci/mmol, Amersham Buchler, Braunschweig, FRG). This probe may also be used in a non-radioactive detection system (B.E.S.T. Probe MZ 1.3, Biotest, Dreieich, FRG). The single-copy DNA probes MS1, MS43 and g3 [6] were labelled with ^{32}P by random oligonucleotide priming [11].

Nylon filters were prehybridized for 4 h in 20 ml of hybridization solution (5 × SSC, 10 m*M* Tris, 2 m*M* EDTA, 0.8% SDS, 5 µg/ml tRNA, 1% nonfat dry milk, pH 7.4) at 62°C. For hybridization, 1×10^6 cpm of labelled probe per ml was added. Hybridization was carried out for 18 h at 60°C. The blots were exposed to Fuji RX film with intensifying screens for varying periods (1–7 days) at −70°C.

Results and discussion

It is known that high-MW DNA is recovered more successfully from muscle tissue than from blood samples [8]. Relatively large quantities of muscle specimens are easily obtainable from cadavers during autopsy, and skeletal muscle tissues are usually less important for histological examinations than other tissues. Therefore, we decided to analyze DNA extracted from postmortem muscles to obtain data on the influences of autolysis and putrefaction on the stability of genomic DNA. During autolysis, the activities of endo- and exonucleases, which degrade DNA in dead cells, are enhanced [8]. In the process of putrefaction, enzymes of bacteria and/or fungi degrade human genomic DNA in dead cells, and produce additional bacterial and fungal DNA.

The high-MW DNA yields (> 15 kb) were analyzed quantitatively according to postmortem periods, sex and causes of death (Table 1). The high MW DNA

Table 1. Yields of high-molecular weight DNA (expressed in mg DNA/g crude tissue) in relation to postmortem periods, sex and causes of death of 33 individuals

		n	Mean ± SD
Postmortem periods:			
1 Day		25	0.357 ± 0.136
2–3 Days		8	0.309 ± 0.090
Sex:	Male	23	0.319 ± 0.135
	Female	10	0.405 ± 0.087[a]
Causes of death:			
Severe injuries		26	0.351 ± 0.115
Injuries and burn		4	0.301 ± 0.165
Burn		3	0.352 ± 0.163
Total mean ± SD			0.345 ± 0.128

[a] $P < 0.05$ vs male individuals

Table 2. Yields of high molecular weight DNA (expressed in mg DNA/g crude tissue) in relation to sex and age

Age (years)	Male		Female		Total	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
10–	6	0.299 ± 0.090	0		6	0.299 ± 0.090
20–	5	0.334 ± 0.102	3	0.318 ± 0.186	8	0.328 ± 0.140
30–	3	0.271 ± 0.057	3	0.410 ± 0.065	6	0.341 ± 0.092
40–	5	0.273 ± 0.069	0		5	0.273 ± 0.069
50–	3	0.427 ± 0.186	3	0.429 ± 0.068	6	0.428 ± 0.140
60–	1	0.421	1	0.577	2	0.499 ± 0.077[a]

[a] $P < 0.05$ vs individuals aged between 10 and 19 years

yields (mg high-MW DNA/g crude tissues) of postmortem periods of 1–3 days were not significantly different. High-MW DNA yields were also not significantly different among victims with different causes of death. On the other hand, the high-MW DNA yields from female samples were slightly higher than those from male samples ($P < 0.05$). Further more, as shown in Table 2, the yields of high-MW DNA increased in relation to the age of the victims. This tendency was more evident in female samples. This may be due to the fact that atrophy of muscles correlates with age and therefore the number of muscle cells and cell nuclei per gram of muscle are increased.

The DNA extracted from muscles after death included approximately 79% high-MW DNA (> 15 kb) (Table 3). Since approximately 21% of total DNA was degraded, it is apparent that autolysis and/or putrefaction affect DNA in muscle tissues even 1 day after death. In 8 of the 33 samples a 1.5-kb band was found on test gels prior to digestion (Fig. 1).

Good RFLP patterns of DNA were obtained from almost all samples by the use of both the multi-locus and the single-locus probes (Table 4). Thus, DNA of

Table 3. DNA yields of muscles before and after storage for 8 days at room temperature at 95%–100% humidity

	Days	*n*	Mean ± SD
mg Total DNA/g muscles:	0	26	0.457 ± 0.190
	8	26	0.533 ± 0.309
mg High-MW DNA/g muscles:	0	26	0.344 ± 0.131
	8	26	0.301 ± 0.187
% High-MW DNA/total DNA:	0	26	78.98 ± 18.08[a]
	8	26	57.61 ± 14.34

[a] $P < 0.005$ vs samples after storage

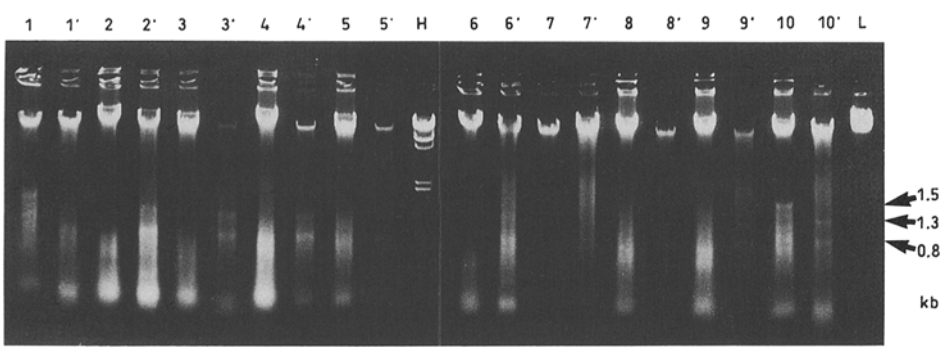

Fig. 1. Electrophoresis patterns of undigested DNA from "fresh" and "old" muscle samples which had been left for 8 days ('), 1 μg of lambda DNA (*L*) and *Hin*dIII-digested lambda DNA (*H*). DNA from old samples included relatively low amounts of high-MW DNA. Note that some fresh DNA showed a 1.5 kb band, whereas some old DNA showed 1.3 and 0.8 kb bands (see *arrows*)

Table 4. Detection of RFLP patterns[a] from fresh and old samples (which had been left at room temperature for 8 days) after autoradiography (varying periods of exposure)

Probe	Sample age	Exposure period (days)	Total number (samples)	Detectable RFLP patterns	
				(*n*)	(%)
MZ 1.3	Fresh	4	30	29	96.7
	Old	4	21	2	9.5
g3	Fresh	2	30	29	96.7
	Old	2	21	3	14.3
		7	21	8	38.1
MS1	Fresh	2	13	12	92.3
	Old	2	13	0	0.0
		7	13	3	23.1
MS43	Fresh	2	12	12	100.0
	Old	2	11	2	18.2
		7	11	3	27.3

[a] The DNA fragment patterns of fresh and old samples were analyzed for the presence of at least six individual-specific fragments for MZ 1.3 and for the presence of two allelic fragments for the single-locus probes

sufficient quality can be obtained from muscle tissues in cases of postmortem age of up to at least 3 days.

Small muscle samples (1–2 g) kept for 8 days at room temperature (20°C) in moist chambers at 95%–100% humidity showed growth of fungi in 9 out of 33 samples after 8 days. As shown in Table 3, a slight increase in the total DNA yield and a slight decrease in the high-MW DNA per gram of tissue were observed after storage for 8 days. This resulted in a statistically significant decrease in the percentage of high-MW DNA/total DNA ($P < 0.005$), indicating an increase in the low-MW DNA fraction in the "old" samples due to degradation. Using test gels, the difference of percentages of high-MW DNA between fresh and old samples was clearly visible (Fig. 1).

When we attempted to digest 10 µg of *high-MW* DNA of old samples by *Hin*fI, more units of *Hin*fI and/or longer incubation periods (2–3 days) were necessary, since the percentage of high-MW/total DNA was decreased in these samples. On test gels, 22 out of 33 DNA samples revealed two distinct bands (1.3 kb and 0.8 kb) before enzyme digestion (Fig. 1). The 1.5-kb band shown in fresh samples was not found. These bands may have been generated by further decay of human genomic DNA or by growth of microorganisms.

In spite of the fact that we could obtain sufficient high-MW DNA from old samples, we often failed to detect RFLP patterns in DNA from old samples, whereas we were able to detect RFLP patterns in more than 90% of the fresh samples (Table 4; Figs. 2 and 3).

Furthermore, we analyzed the quality of DNA from fresh and old muscle samples by use of both a multi-locus minisatellite probe (MZ 1.3) and locus-specific probes (g3, MS1 and MS43). Table 4 summarizes the hybridization results. Almost all DNA from fresh muscles revealed reproducible good RFLP patterns after exposure of the autoradiographs for 2–4 days. In contrast, less

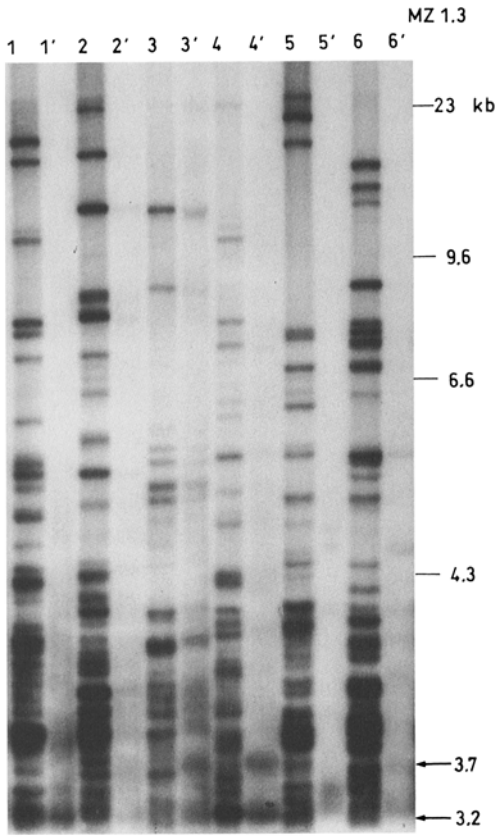

Fig. 2. DNA fingerprinting of fresh and old muscle samples which had been left for 8 days (') using the minisatellite probe MZ 1.3 after autoradiography for 4 days. The fresh samples in lanes 1, 2, 5, 6 revealed clear RFLP patterns, whereas the samples in lanes 3 and 4 already show signs of degradation, since the intensity of DNA fragments > 6 kb is reduced. Of the old samples, only lanes 3' and 6' show fragments that are also present in the corresponding fresh samples. *Arrows* indicate additional 3.2 kb and 3.7 kb fragments not detectable in fresh samples

than 40% of DNA from samples that were stored for 8 days revealed good RFLP patterns after exposure of the autoradiographs for up to 7 days. None of the DNA samples from old muscle specimen on which growth of fungi had been observed showed any RFLP patterns. When the MZ 1.3 probe was used, DNA from old samples often (61.9% of the samples) showed one or two additional bands (3.2 kb and 3.7 kb; Fig. 2), whereas these bands were not present in DNA from fresh samples. Additional bands were not apparent when other probes were used. Since minisatellite probes are not specific for humans, bacterial (or fungal) DNA might cause these additional bands. However, these bands could clearly be distinguished from the human hypervariable fragment patterns, since they were wider than usual and always shorter than 4 kb.

Finally, we compared the results on DNA extraction yields shown in Table 3 (mg total DNA/g muscles; % high-MW DNA/total DNA and mg high-MW DNA/g muscles) to the results of Southern blot hybridization and autoradiography. All mean values of DNA from old samples that clearly showed RFLP patterns were not significantly different from the mean values of DNA from old samples that failed to produce RFLP patterns (data not shown). Also, the DNA yields from a few fresh tissue samples which did not reveal reproducible RFLP patterns were not less than those from other fresh samples. There was no relationship between the results of RFLP detection and sex or age of the victims.

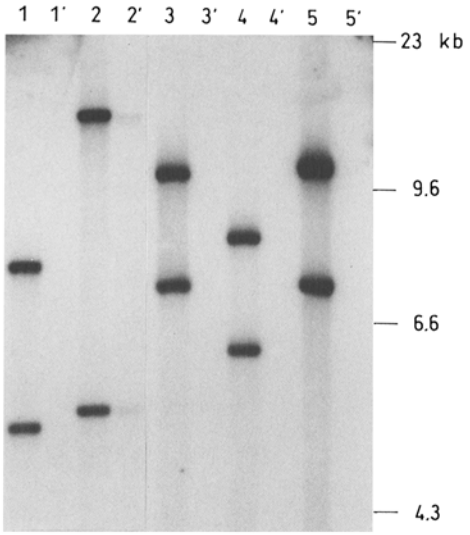

Fig. 3. RFLP patterns of DNA from fresh and old muscle samples which had been left for 8 days (') using the single-locus probe g3 after autoradiography for 7 days. Of the old samples, only lane 2' shows fragments that are also present in the corresponding fresh sample

These results suggest that the quality of DNA from muscle samples could be different from the quantity of DNA, and that the results of Southern blot analysis could not be predicted from the results of quantitative analysis. This may in part be explained by the degradation of human genomic DNA and its substitution by genomic DNA of microorganisms.

Bär et al. [8] examined DNA extracted from several postmortem tissues and showed that good DNA stability was found in the brain cortex, the lymph nodes and the psoas muscle over a period of 3 weeks after death, whereas yields of DNA from blood were not consistent. By Southern blot analysis we could detect RFLP patterns only from $< 40\%$ of DNA from muscle specimens that had been left for 8 days at room temperature. As small strips of muscle tissues were stored in moist chambers at 95%–100% humidity, the putrefaction of tissues might have progressed earlier than usual. In practical cases, the RFLP patterns may be detected after longer postmortem periods, as the muscle tissues are protected by other tissues such as the skin.

Lastly, we compared the RFLP pattern of DNA from a parent with that of DNA from a child using MZ 1.3 (Fig. 4). The hybridization of *Hin*fI-digested DNA revealed that the parent had 16 clear bands > 4.3 kb and the child had 11 bands. Seven bands were common to the parent and the child, resulting in a total band sharing value of 51.8% ($2 \times 7/[16 + 11]$). An average band sharing of 23.8 $\pm$ 7.2% for unrelated individuals and of 59.9 $\pm$ 7.8% for parent-child comparisons has been described using MZ 1.3 [5], suggesting a clear familial relationship between these two individuals. Therefore, DNA fingerprints obtained from muscle samples could be very useful for individual identification in autopsy cases, especially in catastrophic accidents, since muscle tissue can easily be collected from cadavers in sufficient amounts.

In conclusion, our results indicate that the RFLP pattern of DNA could be detected from more than 90% of muscle samples after a postmortem period of up to at least 3 days with subsequent storage of the samples at $-20°C$, and from 40% of muscle specimens after a period of 8 days at room temperature under

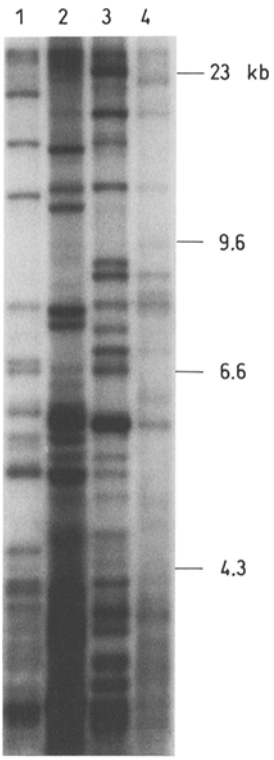

Fig. 4. DNA fingerprint analysis of a parent (*lane 3*), a child (*lane 4*), and two unrelated individuals (*lanes 1 and 2*) using the multi-locus probe MZ 1.3. Band-sharing comparison (see also results and discussion): 1–4: 18.2%; 2–4: 17.4%; 3–4: 51.8%

conditions of high humidity. However, one should be cautious about predicting the success of a DNA fingerprint analysis merely on the basis of the availability of high-MW DNA extracted from tissue samples subjected to autolysis and putrefaction. Our data show that growth of bacteria and fungi on cadaverous tissue may strongly reduce the probability of obtaining human genomic DNA fragment patterns.

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