Properties of chemically oxidized kininogens*

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Kininogens are multifunctional proteins involved in a variety of regulatory processes including the kinin-formation cascade, blood coagulation, fibrinolysis, inhibition of cysteine proteinases etc. A working hypothesis of this work was that the properties of kininogens may be altered by oxidation of their methionine residues by reactive oxygen species that are released at the inflammatory foci during phagocytosis of pathogen particles by recruited neutrophil cells. Two methionine-specific oxidizing reagents, N-chlorosuccinimide (NCS) and chloramine-T (CT), were used to oxidize the high molecular mass (HK) and low molecular mass (LK) forms of human kininogen. A nearly complete conversion of methionine residues to methionine sulfoxide residues in the modified proteins was determined by amino acid analysis. Production of kinins from oxidized kininogens by plasma and tissue kallikreins was significantly lower (by at least 70%) than that from native kininogens. This quenching effect on kinin release could primarily be assigned to the modification of the critical Met-361 residue adjacent to the internal kinin sequence in kininogen. However, virtually no kinin could be formed by human plasma kallikrein from NCS-modified HK. This observation suggests involvement of other structural effects detrimental for kinin production. Indeed, NCS-oxidized HK was unable to bind (pre)kallikrein, probably due to the modification of methionine and/or tryptophan residues at the region on the kininogen molecule responsible for the (pro)enzyme binding. Tests on papain inhibition by native and oxidized kininogens indicated that the inhibitory activity of kininogens against cysteine proteinases is essentially insensitive to oxidation.

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Abbreviations: BAPNA, N-benzoyl-DL-arginine p-nitroanilide; CT, chloramine-T; Me₂SO, dimethylsulfoxide; HK, high molecular mass kininogen; HNE, human neutrophil elastase; HPK, human plasma kallikrein; HPLC, high-performance liquid chromatography; LK, low molecular mass kininogen; MSO, methionine sulfoxide; NCS, N-chlorosuccinimide; PPK, porcine pancreatic kallikrein; pre-HPK, human plasma prekallikrein; PTC, phenylthiocarbamyl; TFA, trifluoroacetic acid.
Oxidation of proteins may occur in the organism wherever the concentration of suitable oxidants increases over the basal level. For example, at the inflammatory foci, a number of reactive oxygen species including superoxide anion and hydroxyl radicals, hydrogen peroxide, hypochlorous acid, etc. are released during phagocytosis of pathogen particles by recruited neutrophil cells (Babior, 1978; Li et al., 1996). On the protein molecules, the methionine and cysteine residues are particularly susceptible to oxidation by these destructive factors (Vogt, 1995). The oxidation of important regulatory proteins may have serious consequences for the homeostasis of the body. Spectacular examples of the effects exerted via protein methionine oxidation to methionine sulfoxide (MSO) include the inactivation of neutrophil elastase-controlling \( \alpha_1 \)-proteinase inhibitor (Johnson & Travis, 1979; Ossanna et al., 1986), \( \alpha_2 \)-macroglobulin (Reddy et al., 1994) and other plasma proteinase inhibitors (Swain & Pizzo, 1988), inactivation of numerous hormones including human growth hormone (Teh et al., 1987), ovine prolactin (Houghten & Li, 1976) or parathyroid hormone (Hong et al., 1986) but, on the other hand, activation of C5 component of human complement (Vogt et al., 1992), etc.

A working hypothesis of this work was that mild oxidation of methionine residues might affect the properties of kininogens, multifunctional proteins involved in kinin formation, blood coagulation, fibrinolysis and inhibition of cysteine proteinases (Colman, 1994; Colman & Schmaier, 1997). Kinins (bradykinin and kallidin), universal mediators of inflammation, are bioactive peptides that are excised from kininogens by serine proteinases called kallikreins (Bhoola et al., 1992). Bradykinin (RPPGFSPFR) is produced from a high molecular mass form of human kininogen (HK) by plasma kallikrein (HPK). Formation of a tight complex of HK with prekallikrein (pre-HPK), a zymogen form of HPK, is essential for its activation (Colman & Schmaier, 1997; Joseph et al., 2001) and the active HPK remains tightly bound to HK. Another, low molecular mass form of kininogen (LK) cannot be processed by HPK but is a source of kallidin (KRPPGFSPFR) upon the action of a tissue-specific kallikrein (Margolius, 1998). In contrast to HK, LK does not possess any distinct kallikrein-binding domain.

In the primary structure of human kininogens, a methionine residue is adjacent to the N-terminus of the internal kallidin sequence. The hypothesis that the oxidation of this particular residue may affect the kinin production by kallikreins was recently tested with model peptides ISLMKRPPGFSPFRSSI and ISL(MSO)KRPPGFSPFRSSI (Kozik et al., 1998). Both types of kallikreins were essentially unable to process the kinin N-terminus in the MSO-containing peptide. It was additionally shown that HK that was oxidized with N-chlorosuccinimide (NCS) could hardly be processed by kallikreins. Unfortunately, no attempt to interpret that finding in terms of protein chemistry was made. Also, the effect of methionine oxidation in LK on kinin formation was not described, and the other possible influences of kininogen oxidation on the physiologically important properties of these multifunctional proteins were not characterized.

In this work we modified both forms of human kininogen with two methionine-oxidizing agents, NCS and chloramine-T (CT), determined the oxidation status of methionine residues in the modified proteins and described in more detail the effects of kininogen oxidation on the kinin formation by kallikreins, on the complex formation between HK and HPK and on the cysteine proteinase (papain) inhibition by kininogens.

**MATERIALS AND METHODS**

**Materials.** N-\( \alpha \)-benzoyl-DL-arginine \( p \)-nitroanilide (BAPNA), bradykinin, cysteine, CT, dimethylsulfoxide (Me\(_2\)SO), human plasma
LK, HPK, kallidin, NCS, papain (type III) and sequanal grade trifluoroacetic acid (TFA) were obtained from Sigma (St. Louis, MO, U.S.A.), single chain HK from human plasma and pre-HPK were from Calbiochem (San Diego, CA, U.S.A.), and high-performance liquid chromatography (HPLC) gradient grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Human neutrophil elastase (HNE) and porcine pancreatic kallikrein (PPK) were kindly provided by Dr. Jan Potempa (Jagiellonian University, Kraków, Poland) and Dr. Hans Fritz (University of Munich, Munich, Germany), respectively. The kinin-containing heptadekapeptide SL(MSO)KRPPGFSPFRSSRI was synthesized at the Molecular Genetics Instrumental Facility (University of Georgia, Athens, GA, U.S.A.) using the standard Fmoc (N-(9-fluorenylethoxycarbonyl) protocol with an Advanced ChemTech MPS350 automated synthesizer. HPLC-grade water was obtained by purification in a MilliQ plus (Millipore, Bedford, MA, U.S.A.) system. Standard chemicals were of ACS grade from Sigma, Merck and Amresco (Solon, OH, U.S.A.).

**Chemical oxidation of kininogens.** Kininogens (HK or LK) at a concentration of 2.5 μM in 0.05 M Hepes buffer pH 7.0, containing 0.15 M NaCl, were incubated with 0.625 mM NCS (added as a freshly prepared solution in the Hepes buffer) for 2 h at 37°C, with occasional stirring. Methionine (solution in the Hepes buffer) was then added to a concentration of 9 mM and the sample was further incubated for 0.5 h at 37°C. In some experiments, NCS concentration was varied (0.07 to 6.25 mM).

Modification of kininogens with CT (Schechter et al., 1975) was performed at the protein concentration of 2.5 μM in 0.05 M Tris/HCl buffer pH 8.5. CT (fresh solution in the same buffer) was added to a concentration of 0.57 mM (or varied within the range of 0.1–4 mM) and the sample was incubated for 20 min at room temperature with occasional stirring. Finally, the reaction was quenched with methionine (9 mM) for 10 min at room temperature.

**Proteolytic processing of native or oxidized kininogens.** Kininogens (1 μM) were treated with kallikreins (0.05 μM) in 0.05 M Hepes buffer pH 7.0, containing 0.15 M NaCl, at 37°C, with occasional stirring. After the desired time, aliquots of the incubation mixture were withdrawn, mixed with one-fifth volume of 1 M HCl and stored frozen until the HPLC analysis for kinin content.

In some experiment, NCS-modified HK was first pre-digested with HNE (0.02 μM) in the Hepes buffer for 2 h at 37°C before addition of HPK (0.05 μM) and further incubation at the same temperature.

**Reversed-phase HPLC of kinins and related peptides.** Bradykinin and kallidin released from kinogens by kallikreins were determined by reversed-phase HPLC analysis on a Supelcosil LC-318 (ODS) column (4 mm × 250 mm) equipped with a Supelguard LC-318 cartridge precolumn (4 mm × 20 mm), in a binary gradient developed between 0.1% TFA in water (solvent A) and 0.08% TFA in 80% acetonitrile (solvent B). The separations were performed at a flow rate of 1 ml/min at ambient temperature, with detection at 215 nm. The gradient applied was linear, starting from 10% B, with a slope of +1.5% B per minute. Solvents were filtered through 0.45 μm Nylon filters (Supelco) and degassed with helium. Samples were centrifuged for 2 min at 10 000 × g at ambient temperature, and placed in autosampler vials from which, typically, 70–90 μl portions were withdrawn for injection. A Shimadzu liquid chromatograph series 10A VP was used that contained: (i) a high-pressure binary gradient forming solvent delivery system based on two LC-10AD VP pumps, (ii) a SIL-10AD VP autosampler, (iii) an SPD-10A VP two-channel variable wavelength spectrophotometric detector, (iv) an SCL-10A VP system controller, and (v) a Class VP (ver. 5.0) hardware/software package for system control and data acquisition/analysis.
Another reversed-phase HPLC system (Kozik et al., 1998) was applied for the identification of the peptide SL(MSO)KRPPGFSPFR in the samples of HK, sequentially digested with HNE and HPK. A LiChro-CART LiChrosphere 100 RP-18 250 mm × 4 mm column (Merck) with a precolumn was applied. Solvent A was 0.1% TFA in water, solvent B was 0.1% TFA in 80% methanol. The separations were performed isocratically (53% B) at a flow rate of 1 ml/min at ambient temperature, with detection at 215 nm. The HPLC instrument used consisted of: (i) a Shimadzu DGU-14A solvent degasser, (ii) a Shimadzu low-pressure quaternary gradient solvent delivery unit (LC-9A HPLC pump and FCV-9AL proportioning valve), (iii) a Knauer model 0263 injector equipped with a 100 μl sample loop, (iv) a Merck-Hitachi L-4000A UV detector, and (v) Schimadzu Class VP (v. 4.0) hardware/software package for pump control, data acquisition and analysis.

**Gel filtration of mixtures of pre-HPK with native or oxidized HK.** Pre-HPK (1.5 μM) was incubated with native or oxidized HK (1 μM) in 0.05 M Hepes buffer pH 7.0, containing 0.15 M NaCl for 1 h and room temperature. Samples were filtered through Millex HV 0.45 μm filters with low protein binding Durapore membrane (Millipore, Bedford, U.S.A.) and subjected to high-resolution gel filtration on a Superdex-200 HR 10/30 column (Pharmacia, Uppsala, Sweden) eluted with 0.05 M sodium phosphate buffer pH 7.0 containing 0.15 M NaCl at a flow rate of 0.5 ml/min at ambient temperature. The chromatographic system used consisted of: (i) a Well Chrom K-1001 HPLC pump (Knauer, Germany), (ii) a Rheodyne 9725 injector equipped with a 200 μl sample loop, (iii) a UV-1 photometric monitor (Pharmacia), and (iv) Chromed PC software (Poznań, Poland) for data acquisition and analysis. The column was calibrated with a Bio-Rad molecular mass standard mixture containing thyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa) and myoglobin (17 kDa).

**Papain inhibition assay.** Papain (Sigma type III), supplied as a solution of 25 mg/ml in 0.05 M sodium acetate buffer pH 4.5, containing 0.01% thymol, was diluted 20-fold into 0.05 M sodium phosphate buffer pH 6.8, containing 10 mM EDTA and 5 mM cysteine. The sample was incubated for 30 min at 37°C for enzyme activation. The activated papain solution (5 μl), 2.5 μM native or oxidized kininogen solution (3–20 μl) and 0.05 M sodium phosphate buffer pH 6.8 with 10 mM EDTA and 5 mM cysteine added to the final volume of 200 μl were mixed in the wells of a 96-well flat-bottom NUNC cell culture microplate (Nalge Nunc, Denmark). The plate was incubated for 30 min at 37°C and then placed in the thermostated chamber of a Bio-Tek PowerWave X–Select scanning microplate reader (Winooski, VE, U.S.A.) set at the same temperature. After 5 min, 4 μl portions of BAPNA solution (50 mM in Me2SO) were added to each well and a continuous monitoring of absorbance at 410 nm was started. The mean reaction rate was calculated from the linear part of the time course of absorbance increase and was used as a measure of the enzyme activity.

**Amino acid analysis.** Amino acid analysis was performed according to protocols described by Cohen et al. (1989). Briefly, protein samples were subjected to gas-phase hydrolysis in 6 M HCl at 115°C for 24 h and the amino acids released were converted to phenylthiocarbamyl (PTC) derivatives. PTC-amino acids were then analysed on a PicoTag 3.9 × 150 mm column (Waters, U.S.A.) using a Waters HPLC system equipped with a 490E-type UV/VIS detector and two 501-type pumps. Modifications of the hydrolysis protocol were applied for the analysis of MSO and tryptophan contents. For determination of MSO, the samples were hydrolyzed in liquid phase using 4 M NaOH at 115°C for 24 h. For tryptophan analysis, the samples were hydrolyzed in liquid phase using 3 M mercaptoethane sulfonic acid at 115°C for 24 h. After hydrolysis the acid was neutralized by addi-
tion of equal volume of 4 M NaOH. In both cases the resulting solutions were then evaporated on a vacuum concentrator, derivatized and analyzed as above.

RESULTS

Determination of the oxidation state of amino-acid residues in chemically oxidized kininogens

The results obtained from amino acid analysis of the samples of HK that were oxidized with 0.625 mM NCS (250 molar excess over protein) and then were subjected to hydrolysis under various conditions, are presented in Table 1. After standard acid hydrolysis, the amino-acid composition of oxidized HK was insignificantly different from that of the native protein. However, this method does not allow one to quantify the content of MSO that is converted with a variable yield to methionine during acid hydrolysis. Hence, samples of NCS-oxidized HK were also subjected to alkaline hydrolysis to separately analyze for MSO and methionine contents. In oxidized HK, the determined amount of MSO was at least 86% of the methionine content of native HK. No residual methionine was detected in oxidized HK. Similar results were obtained upon analysis of oxidized LK (not shown). These data suggest a nearly full conversion of methionine to MSO upon kininogen oxidation with NCS. Only slightly lower methionine oxidation extent (at least 70%) was determined in CT-oxidized HK (not shown). Another method of hydrolysis (in 3 M mercaptoethane sulfonic acid) was used to further confirm the results obtained with NCS and CT oxidants.

Table 1. Changes in amino-acid composition of high molecular mass kininogen after its oxidation with NCS.

HK (2.5 μM) was modified with 0.625 mM NCS in 0.05 M Hepes buffer pH 7.0, containing 0.15 M NaCl, for 2 h at 37°C and then the reaction was quenched with 9 mM methionine for 0.5 h at the same temperature. Samples of modified HK and of native HK were extensively dialyzed against water and hydrolyzed at 115°C, 24 h by three different hydrolytic agents. Amino acid analysis was performed according to the Waters Pico-Tag protocols. Data are means from duplicate determinations.

| Amino acid | Relative change in amino acid content (mol%) after oxidation |
|------------|-------------------------------------------------------------|
|            | standard acid hydrolysis in 6 M HCl | alkaline hydrolysis in 4 M NaOH | hydrolysis in 3 M mercaptoethane sulfonic acid |
| Asx        | 1.03 | 1.10 |
| Glx        | 1.11 | 0.86 |
| Ser        | 1.27 | 1.55 |
| Gly        | 1.18 | 1.09 |
| His        | 0.56 |     |
| Arg        | 0.97 |     |
| Thr        | 0.98 |     |
| Ala        | 0.66 | 0.78 |
| Pro        | 1.25 | 1.21 |
| Tyr        | 1.01 | 0.68 |
| Val        | 1.07 | 0.62 |
| Met        | 0.10 | 0.00 |
| Ile        | 1.06 |     |
| Leu        | 1.04 | 0.90 |
| Phe        | 0.80 | 0.88 |
| Lys        | 0.72 | 0.95 |
| MSO        | 1.94 |     |
| Trp        |     |     |

*Expressed relative to the methionine content in native HK sample subjected to alkaline hydrolysis.
Acid) was applied to determine the content of tryptophan in native and NCS-modified kininogens. About 32% of Trp residues seemed to undergo NCS-mediated modification.

Although the results of amino acid analysis suggested complete oxidation of kininogen methionine residues by NSC, additional analysis was performed to confirm the oxidation state of Met-361, one adjacent to the N-terminus of the internal kallidin sequence in kininogen. A sample of NCS-oxidized HK was first digested with human neutrophil elastase (HNE) that had previously been reported to cleave the peptide bond of kininogen after Ile-358 (Sato & Nagasawa, 1988; Imamura et al., 2002). After further action of HPK that should expose the kinin C-terminus, the sample was analyzed in a specifically dedicated reversed-phase HPLC system. On the chromatograms obtained (Fig. 1) a species is visible with the retention time identical to that of the peptide SL(MSO)KRPPGFSPFR that is generated upon a short treatment of the peptide SL(MSO)KRPPGFSPFRSSRI with HPK. These data suggest, at least qualitatively, that Met-361 is oxidized in NCS-oxidized HK.

**Kinin formation from chemically oxidized kininogens by plasma- and tissue kallikreins**

Time courses for bradykinin production from native and NCS-oxidized HK by HPK and for kallidin formation from native and NCS-oxidized kininogens (HK and LK) by a tissue kallikrein (PPK) are shown in Fig. 2. Kinin production was strongly quenched after substrate oxidation. However, this effect was rather moderate in the tissue kallikrein-dependent system as compared to the nearly complete abolishment of bradykinin production by HPK from NCS-oxidized HK. As shown in Fig. 3A, quite a low molar excess of the oxidizing agent (28-fold over kininogen, i.e. 3.5-fold over methionine residues) was sufficient to lower the kinin yield by about 50%. At these low NCS concentrations no difference between the actions of plasma and tissue kallikreins on the oxidized substrate was detectable. As NCS excess increased, the kallidin yield in the tissue-kallikrein system seemed to stabilize at the level of about 30% of that for native kininogen (HK or LK); however, the bradykinin formation from HK by HPK fell down to a really residual yield at NCS concentration higher than 2 mM. When CT was used for kininogen oxidation, the dependence of kinin yield on the oxidant concentration (Fig. 3B) was quantitatively similar between the HK-HPK and LK-PPK systems. Moreover, it essentially matched the characteristics obtained for the tissue kallikrein-dependent processing of NCS-modified kininogens; at the highest CT concentration the kinin production reached 30% of that from native substrate.
Pre-HPK binding to native and oxidized HK

The results of high-performance gel filtration of mixtures of pre-HPK with native or oxidized HK are presented in Fig. 4. Pre-HPK (molecular mass 85 kDa) was applied in a 1.5 molar excess over kininogen (molecular mass 120 kDa). Analysis of the samples containing pre-HPK with native HK revealed two peaks with apparent molecular masses of 220 kDa and 85 kDa. The first one seemed to represent a complex of both components, the second was of the prekallikrein excess. Essentially the same elution profile was obtained for the mixtures of pre-HPK with CT-oxidized HK, clearly indicating that the modified kininogen retained full capability of pre-HPK binding. In sharp contrast, an insignificant amount of the 220 kDa species could be detected upon analysis of mixtures of pre-HPK with NCS-oxidized HK. Thus, NCS-modified HK seemed to be unable to form a stable complex with pre-HPK.

Inhibition of papain by native and oxidized kininogens

The characteristics of papain inhibition (Fig. 5) were essentially indistinguishable between native kininogens (HK or LK) and kininogens subjected to oxidation with NCS at submillimolar concentrations. The inhibitory capability was slightly impaired only at the NCS concentrations higher than 6 mM, i.e. exceeding 10-fold the levels used in all experiments described in the previous sections. Similarly, a complete resistance of the papain-inhibitory activity of kininogens toward oxidation by CT was determined (not shown). In control experiments it was shown that under
conditions required for papain activation (5 mM cysteine, 37°C, 1 h) the model peptide SL(MSO)KRPPGFSPFRSSRI could not be converted to SLMKRPPGFSPFRSSRI in any significant yield. Hence, the possibility of a reversal of kininogen oxidation during the papain inhibition assay was definitely excluded.

**DISCUSSION**

Reactive oxygen species that may occur in the living organism have an oxidative potential sufficient to convert methionine residues to MSO (Vogt, 1995). Further oxidation to methionine sulfone is unlikely to proceed in vivo. Of the chemicals commonly used for protein oxidation for the purposes of structural studies, NCS and CT were reported to be satisfactorily specific toward methionine (Schechter et al., 1975) and to be effective at submillimolar concentrations that might be sufficiently non-destructive to the protein native conformation. Both reagents oxidize methionine to MSO; hence, in terms of the product formed, the modified protein may have some biological relevance. Moreover, chemical properties of CT resemble to some degree those of numerous chloramines that may be formed in vivo by the action of hypochlorous acid on biological amines. Obviously, both NCS and CT also oxidize cysteine residues if present in the protein being modified. However, all cysteine residues of kininogens are involved in disulfide bridges so that no free sulfhydryl group occurs in the protein molecule (Colman, 1994). Another, although physiologically perhaps less relevant, side reaction of NCS is the modification of tryptophan residues (Schechter et al., 1975). This type of mod-
ification is less likely when CT is used for protein oxidation (Schechter et al., 1975).

Methionine residues are located at very strategic points on the kininogen molecule (Colman, 1994). Two of them (Met-357 and Met-361) occur in the small kinin-containing domain D4; the latter residue is directly adjacent to the internal kinin sequence (residues 362–371). Previous studies on model peptides suggested that conversion of the hydrophobic Met-361 residue to the more polar MSO should quench the release of kinin N-terminus by both types of kallikreins. In this work we qualitatively confirmed the oxidation of Met-361 in NCS-modified kinogens. As judged from direct analysis of amino-acid composition of NCS-modified kinogen, nearly all methionine residues seemed to be oxidized by this reagent. Hence, the oxidation of Met-361 might be the primary structural reason of the ineffective processing of NCS- and CT-oxidized kinogens by kallikreins. In this work, the quenching of kinin release was confirmed for all kininogen-kallikrein combinations except for the physiologically insignificant LK-HPK pair. This quenching effect was observed at a relatively low molar excess of the oxidizing agents over the protein, suggesting an exceptionally high susceptibility of Met-361 toward oxidation. This high reactivity is rather expected as Met-361 should be located in a loop that must be sufficiently exposed to the solvent to be accommodated by the active site of kallikrein.

No significant difference in the magnitude of the quenching effect on kinin release was detected between NCS- and CT-oxidized kinogens (HK or LK) in the process performed by tissue kallikrein. This indicates that the smaller overall extent of methionine oxidation by CT is not essential for the impairment of the kinin-precursor property of oxidized HK and LK.

The clearly much stronger effect of NCS-oxidation of HK on bradykinin release from this substrate by HPK may be through a possible impairment of HPK binding to HK. Formation of this tight complex is a prerequisite for the effective catalytic action of HPK on HK (Bhoola et al., 1992; Colman & Schmeier, 1997). We found that NCS-oxidized HK had no capability of pre-HPK binding, in sharp contrast to CT-oxidized HK that bound pre-HPK as tightly as native HK. In the HK molecule, three methionine residues (Met-554, Met-555 and Met-613) flank the region responsible for pre-HPK binding. Although neither is located within the binding region (residues 565–595), their conversion to polar MSO residues may have a significant effect on the overall conformation of the D6 domain. Then, however, some effect of CT-modification of HK on pre-HPK binding should probably be detectable. Unfortunately, a single tryptophan residue (Trp-569) is located just in the contact region. Modification of this residue by NCS, not just oxidation of the Trp side chain but even cleavage of the peptide bond adjacent to this residue, could be expected to completely cancel the possibility of this protein–protein interaction. Moreover, another tryptophan residue (Trp-601) is in the vicinity of the pre-HPK region at the C-terminal side. We detected a significant modification of tryptophan residues in NCS-oxidized HK, although no attempts to assign this modification to specific residues in the pre-HPK region were made. Anyhow, it should be emphasized that the tight complex with pre-HPK is the natural form of HK and in this complex the residues present in/at the binding region are expected to be protected from oxidation under mild conditions. Thus it is rather oxidation of HK by CT that may be considered as a model of oxidative quenching of bradykinin formation in vivo.

Another activity of kinogens (both HK and LK), inhibition of cysteine proteinases, was found insensitive toward oxidation by NCS or CT when papain was used as a model enzyme. Met-160 is located at the N-terminal part of the inhibitory domain D2 and Trp-204 at the C-terminal end of this domain. Some decrease of the papain-inhibiting capability was only
observed in HK oxidized with exceptionally high NCS concentration. Although under those conditions Trp-204 might be modified, non-specific denaturation of some kininogen molecules was more likely.

Taken together, our results indicate that mild oxidation of kininogens, via conversion of methionine residues to MSO residues, results in a selective quenching effect on kinin-precursor properties of these multifunctional proteins. Other biologically essential kininogen functions, interaction of HK with plasma prekallikrein (and probably the entire pro-coagulation function of HK) and inhibition of cysteine proteinases by both HK and LK, may be preserved. A biological significance of those findings remains yet to be established. It is possible that quenching of kinin release at the inflammatory foci due to the action of reactive oxygen species released by phagocytic neutrophils may help to stop kinin production at late stages of inflammatory reaction and to initiate the tissue restoration.

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