

Characterization of urinary bikunin and its role in urolithiasis Fouad Atmani

Un nouveau inhibiteur urinaire de la formation de l'oxalate de calcium a été isolé de l'urine de sujets sains. La même protéine isolée de l'urine d'individu ayant développé des calculs rénaux, montre moins d'activité inhibitrice que les témoins. Cet inhibiteur a aussi été isolé de l'urine provenant des reins de rats et de bovins. Il a été identifié par la suite comme la bikunine urinaire, la chaîne légère alpha de l'inhibiteur inter- α (I α I). Sa présence dans les matrices organiques des cristaux et des calculs rénaux indique qu'il pourrait jouer un rôle clé dans la lithiagénèse. En utilisant un modèle de rat néphrolitique, il a été démontré que le gène de la bikunine peut être exprimé dans les reins. L'ensemble de ces résultats suggère que les reins possèdent un système de défense contre la cristallisation de l'oxalate de calcium et la formation des calculs, qui est principalement gouverné par les protéines de la famille des I α I, en particulier la bikunine urinaire.

A new urinary inhibitor of calcium oxalate formation was isolated from the urine of healthy subjects. The same protein isolated from the urine of stone formers showed less inhibitory activity than that of controls. This inhibitor was also isolated from the urine of rats and bovine kidneys. It was identified later as urinary bikunin, the light chain of inter- α -inhibitor (I α I). Its presence in organic matrices of crystals and kidney stones indicates that it may fulfill a directive role in lithogenesis. By using a nephrolithic rat model, it was demonstrated that bikunin gene can be expressed in the kidney. Altogether, the data suggests that kidneys possess a defense system against calcium oxalate crystallization and stone formation which is mainly driven by proteins of I α I family, especially urinary bikunin.

Key words

Nephrolithiasis, Calcium oxalate crystallization, Kidney stones, Bikunin.

Introduction

Biological mineralization is an important biological processes in living organism. It can be a normal phenomenon such as the formation of bone and teeth or a pathological process such as the formation of renal stones (urolithiasis). The present review will be focused on the latter, which involves physico-chemical steps, namely crystallization. Crystallization begins with supersaturation leading to nucleation, followed by crystal growth and aggregation. In this regard, it is recognized that kidneys produce supersaturated urine with respect to calcium oxalate (CaOx), a predominant constituent of most human kidney stones. Therefore, under favorable conditions, CaOx may readily precipitate in the urine and lead to stone formation in the urinary tract. Fortunately, the urine contains several macromolecules that efficiently prevent such concretion in healthy people (Atmani et al., 1998). Among these substances, the urinary glycoprotein, bikunin, has drawn attention over the last decade.

From uronic-acid-rich protein to bikunin

After showing that urine can inhibit cartilage calcification, Howard et al. (Howard et al. 1967) were the first to isolate from urine two polypeptides endowed with the capacity to inhibit calcification. Numerous studies successively demonstrated that urine contained non-dialysable substances with a molecular weight greater than 10 kDa, which had an in vitro inhibitory activity toward CaOx crystallization. In order to identify the nature of these substances, Nakagawa et al. (Nakagawa et al. 1978) conducted a series of experiments leading to isolation of a urinary glycoprotein with a molecular weight of approximately 12 kDa. According to these investigators, this protein named nephrocalcin later is the main inhibitor of CaOx crystallization (Nakagawa et al. 1983). Subsequently, other studies including ours, have demonstrated that several other proteins exhibit a significant inhibitory activity regarding CaOx crystallization (Atmani et al. 1993a, Sørensen et al. 1990). Indeed, we have isolated and purified a glycoprotein from the urine of healthy subjects using three chromatographic procedures (Atmani et al. 1993a). It has a molecular weight of approximately 35 kDa as estimated by SDS-polyacrylamide gel electrophoresis. Its amino acid composition revealed

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that glutamic and aspartic acids represent 24% of total amino acid content (Atmani et al. 1993b). Because of its high content of uronic acid, this glycoprotein is called uronic-acid-rich protein (UAP). A similar protein was isolated from rat urine and contained the same amino acid composition as human UAP (Atmani and Khan, 1995). The first 18th N-terminal amino acid from the sequence of both human and rat UAP showed a structural identity with bikunin, a fragment of inter- α -inhibitor (I α I). In addition, using a polyclonal I α I antibody, positive Western blot indicated a relationship between UAP and I α I. Subsequently, UAP was confirmed to be urinary bikunin, based on its reactivity to a bikunin antibody (Atmani et al., 1996a).

The origin of bikunin

Amino acid sequencing and immunoblotting showed that UAP is bikunin, the light chain of I α I, a large macromolecule that comprises also two heavy chains, H1 and H2 (Salier et al., 1987). Moreover, bikunin is encoded by a common gene that codes also for α 1-microglobulin (α 1-m) (Kaumeyer et al., 1986). As soon as they are synthesized, a cleavage occurs and both α 1-m and bikunin are released separately (Pierzchalski, 1992). In this regard, bikunin can be found in free form or bound to different complexes related to I α I. The free form can easily be excreted in urine through glomerular filtration due to its low molecular weight. Interestingly, the three chains constituting I α I are held together in a unique complex intermediately by way of a carbohydrate moiety sensitive to chondroitinase digestion (Jessen et al., 1988, Balduyck et al., 1989).

It is generally recognized that I α I and its derivatives are synthesized mainly in the liver and excreted in plasma. This finding was confirmed specifically by molecular biology (Salier et al., 1987). However, Kastern et al. (Kastern et al., 1986) have shown that α 1-m precursor, which codes also for bikunin, is present in high level in liver and kidneys of rats. This result leads to the hypothesis that bikunin might be synthesized in kidneys. Using reverse transcription-polymerase chain reaction (RT-PCR) technique, it has been demonstrated that bikunin gene is expressed not only in liver but in other human tissues including the kidney (Mizushima et al., 1998). We recently confirmed this finding in experimentally induced CaOx urolithiasis in rats (Atmani et al., 1999).

Bikunin in urolithiasis

Sørensen et al. (Sørensen et al. 1990) have isolated from urine of healthy subjects a 40 kDa protein that had an important inhibitory activity regarding CaOx crystallization. The authors showed that this inhibitor is related to I α I since their first 16th N-terminal amino acid are identical. In our laboratory we have shown that UAP, identified later as urinary bikunin (Atmani et al., 1996a), is a potent inhibitor of CaOx crystallization (Atmani et al. 1993a-b). We have also demonstrated that urinary bikunin inhibits efficiently CaOx crystal nucleation and aggregation (Atmani and Khan, 1999a). However, the inhibitory activity of this protein is reduced in the urine of stone formers compared with normal controls (Atmani et al., 1994). This finding allowed proposing that the inhibitor derived from stone formers might be structurally abnormal. A recent study has shown that the concentration of urinary bikunin is significantly reduced in CaOx stone formers compared to healthy volunteers (Médétognon-Benissan et al., 1999).

Altogether, data indicate that there is a close association of I α I members with urolithiasis. In order to clarify this relation we conducted two separate studies aiming first to verify whether bikunin and other members of I α I family are synthesized in the kidneys (Atmani and Khan, 1999b) and second to compare the immunohistological distribution of I α I related proteins and study the expression of bikunin gene in the kidneys of normal and nephrolithic rats (Atmani et al., 1999). The results showed that proteins extracted from homogenized bovine kidney, after they were applied successively to three chromatographic steps on DEAE-Sephacel, Sephacryl S-300, and Mono Q column, contained a 125 kDa protein which cross-reacted with anti-I α I antibodies. This protein inhibited CaOx crystallization efficiently. According to its molecular weight and immunoreaction with anti-I α I antibody, the 125 kDa protein could be the pre- α -inhibitor. The latter is known to encompass a heavy chain and bikunin, which may explain its inhibitory activity against CaOx crystallization. Furthermore, examination of kidneys from normal rats showed the expected pattern of I α I immunoreactivity in the proximal tubules only (Atmani et al., 1999, and Ødum, 1989). In the nephrolithic rats, in addition to the proximal tubules, I α I immunoreactivity was intensively localized in the renal tubular segments of the corticomedullary junction (Atmani et al., 1999). In order to ascribe the renal epithelial cell as the site of I α I expression, total mRNA was extracted from different parts of the

kidney and from liver as control. Amplification of the bikunin gene by RT-PCR allowed to identify an intense band at 1.4 kb, corresponding to the bikunin gene appeared in both liver and kidney of nephrolithic rats. This suggests that the bikunin gene is upregulated in response to hyperoxaluria and CaOx crystals deposition (Atmani et al., 1999). This finding differs from that of Salier et al. (Salier et al., 1993) who demonstrated that αI and its related proteins are synthesized only in the liver. This discrepancy may be explained by the fact that Salier and colleagues studied the expression of these proteins in normal rat kidneys. Our laboratory has also shown an increase in bikunin gene expression in LLC-PK₁ and MDCK cells exposed to oxalate and CaOx crystals (Ida et al., 1997). These two cell types were used as representative of epithelia lining the proximal tubule and collecting ducts respectively. Based on results described here and elsewhere, we hypothesize that bikunin gene is upregulated by hyperoxaluria and calcium oxalate crystal deposition.

Finally, it must be pointed out that regardless of their mineral content, all urinary stones contain a network of organic material referred often to as organic matrix. It is distributed throughout the stone in a random manner or according to a pattern of fibrous concentric and radial striations. The ubiquity of this matrix and its high protein content (Boyce, 1968), suggest that these proteins may fulfill a directive role in stone formation. Studies to understand the interaction between the organic matrix, especially proteins, and mineral phase in the early stage of the crystallization are therefore mandatory. In this regard, our efforts have been aimed at the study of bikunin and other proteins belonging to the αI family (Atmani et al., 1996b, Atmani et al., 1998). The presence of these proteins in crystal matrix indicates the possibility that they may be involved in modulating crystallization either individually or in concert with each other to give a resultant effect.

Conclusions and perspective

Although treatments have substantially improved, it is generally argued that better strategies for preventing kidney stones need to be developed. Accordingly, it is strongly recommended to take in consideration the role that macromolecules can play in preventing stone formation. In this regard, our results demonstrated that urinary bikunin inhibit efficiently nucleation and aggregation of caox crystallization. furthermore, the bikunin synthesis is stimulated during hyperoxaluria an/or caox crystal deposition in kidney. therefore, the

general issue of how bikunin can be used in kidney stone therapy needs to be adressed. undoubtedly, the ongoing research in this area will surly participate in our general understanding the mechanisms of lithogenesis and how can be prevented or at least reduced.

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