Clinical Significance of Pro–B-Type Natriuretic Peptide Glycosylation and Processing

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Since the principal discovery of the cardiac natriuretic peptide hormones about 25 years ago, a large amount of research has identified 2 peptides derived from pro–B-type natriuretic peptide (proBNP),2 BNP and N-terminal–proBNP (NT-proBNP), as useful plasma markers in cases of heart failure (HF) and other cardiac diseases. Our present understanding of proBNP processing and the structural biochemistry and metabolism of circulating proBNP-derived peptides, by contrast, are still far from complete. In particular, posttranslational maturation and metabolism are still poorly characterized. In this issue of Clinical Chemistry, Semenov et al. describe important results that may substantially improve our current understanding of the regulation of proBNP processing (1). Like many other hormones, BNP is derived from a prohormone. The precursor proBNP hormone is encoded by a separate gene, NPPB (natriuretic peptide precursor B), which has been assigned to chromosome 1 in humans. Its transcription has been shown to be regulated by cardiac-specific gene regulators, such as GATA, the muscle-CAT binding site, and activating protein 1/cyclic adenosine monophosphate response element–like elements (2). These cis elements have since been found to be the molecular targets of many different clinically relevant stimuli that lead to basal and inducible regulation of the NPPB gene. Transcription of the human NPPB gene can be activated through various proinflammatory and hypertrophic stimuli, such as mechanical stretch, ischemic injury/hypoxia, endothelin–1, angiotensin II, interleukins, and adrenergic agonists (2). The 5′ flanking region of the NPPB gene contains acute-phase regulatory elements, and the expression of this gene is induced with the rapid kinetics of an early-response gene. In contrast to A-type natriuretic peptide (ANP), it is believed that most BNP regulation is carried out during gene expression, with most BNP being synthesized in bursts of activation from physiological and pathophysiological stimuli, when peptide secretion occurs. Only limited amounts of proBNP and processed BNP coexist in the secretory granules of the human atrial myocardium (3). The available information on posttranslational proBNP processing is partially based on indirect observations (see Fig. 1). Translation of the NPPB gene produces an initial gene product, precursor proBNP 1–134, which undergoes rapid removal of a 26–amino acid signal peptide in the sarcoplasmic reticulum during translation before synthesis of the C-terminal part of the prohormone sequence has been completed. This cleavage yields a prohormone of 108 amino acid residues, proBNP 1–108. Prohormone convertases such as furin and corin (4, 5) may subsequently cleave proBNP to release 2 portions: the 76-residue amino-terminal portion (NT-proBNP 1–76) and the biologically active 32-residue molecule, BNP 1–32. The cleavage site is located between amino acid residues 76 and 77 (−Leu72–Arg73–Ala74–Pro75–Arg76 ↓ −Ser77−). In vitro experiments and cell-based assays have demonstrated that furin and corin are able to process proBNP. The significance of each protease for in vivo proBNP processing is still controversial, and other convertases may be involved as well. Furin is a membrane-associated calcium-dependent serine endopeptidase in the yeast Kex2 family. It is localized in the trans-Golgi networks and recycles between the trans-Golgi networks and plasma membranes. Whereas corin is uniquely distributed in the myocardium, furin is found in various tissues; however, the furin gene is highly expressed in hypertrophic cardiomyocytes. proBNP possesses a furin-cleavable sequence at its processing site (−Arg73–Ala74–Pro75–Arg76 ↓ −Ser77−). Corin is a membrane-bound type II transmembrane serine peptidase that is produced at high concentrations in the heart and is up-regulated in hypertrophic cardiomyocytes and failing myocardium. In the presence of adequate stimuli, corin cleaves proANP into the N-terminal proANP 1–98 and the C-terminal ANP 99–126, which are then released into the blood; however, corin has been shown to be capable of processing proBNP as well. Cardiac secretion of BNP and NT-proBNP has been demonstrated by sampling blood from the coronary sinus, and the
The circulation of intact proBNP in the blood has also been described (6). In addition, BNP and NT-proBNP have been recognized to be modified into a mixture of various fragments (7, 8). A substantial portion of the immunoreactive BNP circulates in humans as protease-degraded fragments, and its N-terminal end seems to be particularly susceptible to enzymatic degradation. Proteolytic fragmentation of NT-proBNP 1–76 at both the C- and N-terminal ends of the molecule to produce smaller immunoreactive molecules in the circulation has also been reported. Commercial assays for BNP and NT-proBNP measure a mixture of cleaved and uncleaved analytes, as well as proBNP to varying extents (9). Initial studies based on fractionating of plasma samples by chromatography and testing the fractions for BNP immunoreactivity, as well as later studies that used western blot analysis of plasma samples from HF patients, have revealed the presence of both low molecular weight and high molecular weight forms of immunoreactive BNP and NT-proBNP (7, 10). Immunoreactive NT-proBNP and BNP in plasma were shown to elute at a position corresponding to a much higher molecular weight than expected by chromatography of the synthetic proBNP and NT-proBNP standards. This result was confirmed in western-blotting studies. The presence of a leucine zipper–like motif in the proBNP sequence led to speculations that proBNP and its N-terminal fragments form oligomers. This process is unlikely to occur in vivo, however, and proBNP has been shown to circulate as a monomer (11). Recently, in vivo glycosylation of proBNP and NT-proBNP was identified as an explanation for this phenomenon, because the synthetic standards used in these experiments were nonglycosylated (12). proBNP may be posttranslationally glycosylated to a varying degree at several sites (Thr36, Ser37, Ser44, Thr48, Ser53, Thr58, Thr71) in its N-terminal region (12). The central portion of human circulating NT-proBNP (amino acid residues 28–56) is also glycosylated, but the C-terminal end (residues 61–76) of the molecule is almost free of O-linked glycans (13). When plasma samples were deglycosylated, western-blotting bands were found at regions corresponding to the nonglycosylated proBNP and NT-proBNP standards (10, 12).

The clinical relevance of proBNP glycosylation is an obvious burning question. Recently, antibody selection for NT-proBNP immunoassays has been proposed as potentially important (13). The central part of NT-proBNP is the most stable region of the molecule; however, glycosylation may occur in this region (12, 13). All commercially available NT-proBNP assays approved for routine diagnostic use are based on the same antibodies (i.e., from Roche Diagnostics). These antibodies were originally polyclonal antibodies directed against epitopes 1–21 and 39–50. Most recently, monoclonal antibodies directed against epitopes 27–31 and 42–46 have been used. Glycosylation of the middle portion of NT-proBNP may affect binding of the antibody directed against epitope 39–50 or 42–46 (13), potentially leading to an underestimation of the “real” concentration of circulating NT-proBNP. The clinical relevance of this possible interference with antibody binding remains to be established, however, because clinical results obtained with the current routine NT-proBNP assays are excellent and comparable with those for BNP. A substantial improvement in the clinical performance of NT-proBNP measurements with assays that avoid the detection of epitopes that could be affected by glycosylation remains to be demonstrated. Antibodies directed against
epitopes at the N- or C-terminal end of NT-proBNP, however, may lead to reduced stability of NT-proBNP in vitro, compared with the stability of NT-proBNP with currently commercially available assays, which use antibodies directed against epitopes in the more stable central region of the molecule. Occasionally, however, a high degree of glycosylation in an individual patient may be responsible for unexpectedly low NT-proBNP values with commercial assays that use antibodies directed against the central region of NT-proBNP. Differences in the glycosylation profiles in region 61–76 of proBNP and circulating NT-proBNP have led to speculations that the glycosylation status of the region near the cleavage site may play a role in regulating proBNP processing by shielding the cleavage site. A study published in this issue (1) investigated the influence of proBNP glycosylation on in vitro proBNP processing and found evidence for a role of Thr71-bound O-glycan in suppressing furin processing of proBNP. Posttranslational modifications of proteins, such as phosphorylation, acetylation, and glycosylation, are established mechanisms for the regulation of protein function. Patients with advanced HF appear to be resistant to the physiological actions of natriuretic peptides, but therapeutically administered BNP, surprisingly, is effective in treating acutely decompensated HF. There is accumulating evidence that differently activated proBNP values with commercial assays that use antibodies directed against the central region of NT-proBNP. Differences in the glycosylation profiles in region 61–76 of proBNP and circulating NT-proBNP have led to speculations that the glycosylation status of the region near the cleavage site may play a role in regulating proBNP processing by shielding the cleavage site. A study published in this issue (1) investigated the influence of proBNP glycosylation on in vitro proBNP processing and found evidence for a role of Thr71-bound O-glycan in suppressing furin processing of proBNP. Posttranslational modifications of proteins, such as phosphorylation, acetylation, and glycosylation, are established mechanisms for the regulation of protein function. Patients with advanced HF appear to be resistant to the physiological actions of natriuretic peptides, but therapeutically administered BNP, surprisingly, is effective in treating acutely decompensated chronic HF. There is accumulating evidence that different molecular forms of proBNP-derived peptides have differential biologic activities in HF (14, 15). NT-proBNP 1–76 has no biologic activity, and proBNP 1–108 has no or markedly reduced biological activity in vitro compared with BNP (6- to 8-fold less potent than BNP in vascular endothelial and smooth muscle cells). proBNP and NT-proBNP showed no effects on human cardiac fibroblasts and cardiomyocytes. There is evidence that the ratio of circulating proBNP to BNP varies from patient to patient and is disease dependent. The substantially less hormonally active proBNP may be the major immunoreactive form of BNP in patients with severe HF (6, 14). The precise mechanisms leading to this phenomenon are currently unknown, but impairments of proBNP processing may be involved. Thr71 O-glycosylation may impair furin processing of proBNP into BNP and NT-proBNP (1). A limitation of the study by Semenov et al. is that only furin processing of proBNP was investigated; corin, the other candidate enzyme for proBNP processing, was not investigated. Thus, no definitive conclusions about the relevance of proBNP glycosylations for in vivo proBNP processing in men are currently possible, but the authors are to be congratulated for the first experimental work on this topic, which should encourage future studies on the regulation of proBNP processing.

**References**


