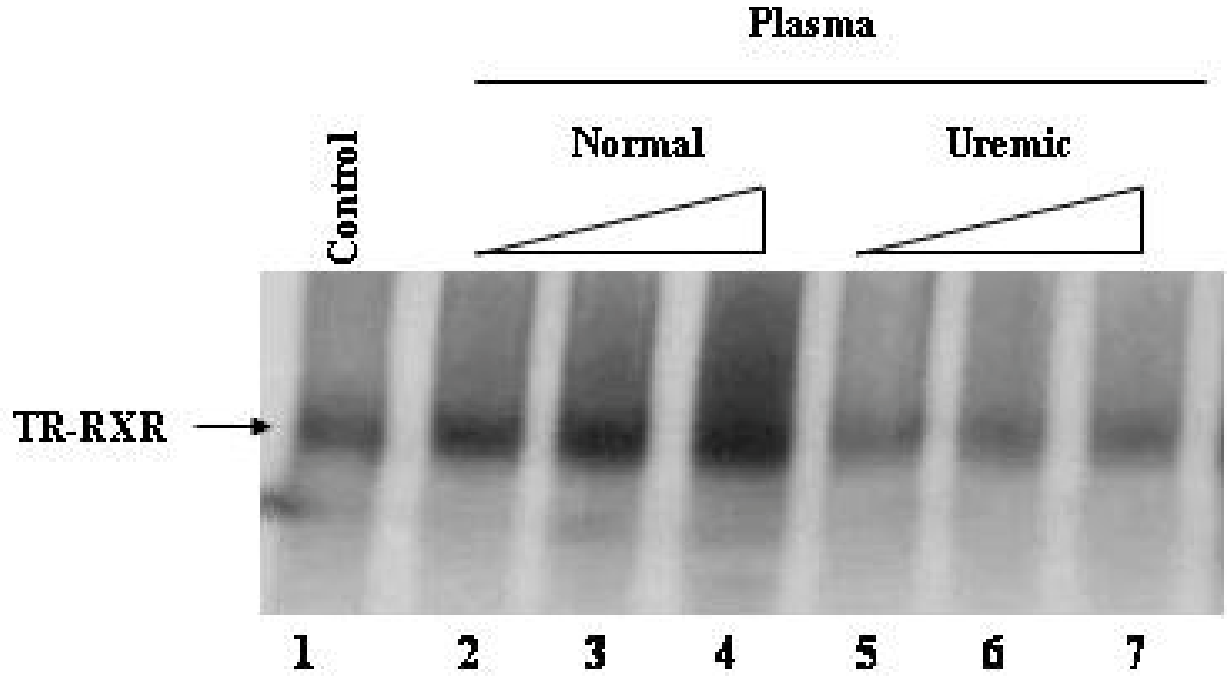

Figure 1
Uremic plasma reduces hTRβ1-hRXRα complex formation on DR-4. Gel Shift experiments were performed using in vitro translated [^{35}S] hTRβ1, cold hRXRα and DR-4. [^{35}S] hTRβ1 was treated with T_3 10^{-7}M for 30 min at 4°C and then incubated without (Control – lane 1) or with increasing volumes (0.5, 1.0, 2.0 μL) of normal (lanes 2–4) or uremic (lanes 5–7) plasma for 30 min at 4°C. Cold DR-4 type TRE (5'- AGCT TC **AGGTCA** CAGG **AGGTCA** GAG - 3'), cold hRXRα, and nonspecific DNA poly (dIdC) were subsequently added and incubated for 20 min.

To exclude the possibility that the inhibition of hRXRα-hTRβ1 binding to DNA induced by uremic plasma could be due to proteolytic degradation of TRβ1, we incubated ^{35}S-labeled TRβ1, at the same conditions as in the gel-shift experiments, with normal or uremic plasma at 4°C, for thirty minutes. ^{35}S-labeled TRβ1 samples were then analyzed by SDS-PAGE. As expected, the major translation product of TRβ1 was 53 kD and incubation of theses products with normal or uremic plasma did not modify the translated [^{35}S]TRβ1 (Figure 2). These results indicated an absence of uremic proteolytic activity that might be involved in the decrease of TR-RXR complex formation on DNA.

Hemodialysis improves hTRβ1-hRXRα binding to DR-4

It is yet unknown why uremic plasma diminished TRβ1-RXRα binding to DNA, when compared to non-uremic plasma. The observed effect could be ascribed either to a lack of some factor(s) typically present in normal plasma or to the presence of some inhibitory products present in uremic plasma. To test the second hypothesis, we analyzed the influence of uremic plasma, collected before and after hemodialysis, on TRβ1-RXRα-DR-4 complex formation using gel-shift assays. In these experiments, [^{35}S] TRβ1 was incubated with normal or uremic plasma, collected before (pre-HD) or 4 h after hemodialysis (post-HD). As shown in Figure 3, uremic plasma collected before HD (lanes 5–7) decreased the binding of hTRβ1-hRXRα to DNA (DR-4), relative to normal plasma (lanes 2–4). However, when these receptors were pre-incubated with uremic plasma collected from the same patient after hemodialysis (lane 8–10), an important improvement of hTRβ1-hRXRα binding to DR-4 was observed. Although hemodialysis improved complex formation, it did not completely recover the inhibition caused by uremic plasma. Densitometry analysis demonstrated that