

Study of protein-DNA interactions by surface plasmon resonance (real time kinetics)

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Submitted March 27, 1991

The BIA core system from Pharmacia uses surface plasmon resonance (SPR) for the detection of macromolecules interactions and appears already as a powerful tool to study the binding kinetics of antibodies to antigens (1). SPR is a phenomenon which occurs between incoming photons and the electrons in the surface of a thin metal film mounted on a glass support. At a specific angle of incident light, energy is transferred to the electrons in the metal surface causing the reflecting light to disappear. This angle of non reflectance, the resonance angle, changes as the mass concentration in the vicinity of the metal surface changes. Therefore, binding or dissociation of interacting molecules, reflected by changes in mass concentration, can be measured with 'BIA core' system (1–3).

Here we show that the above method can be also used to study the interaction of DNA with proteins in crude preparations using the same binding conditions as for the classical gel mobility shift assay (4). A synthetic double stranded oligonucleotide (40 mer) derived from the binding site of the nuclear repressor R1 from chicken liver (4) was biotinylated at its 3′ end and linked to the surface of the biosensor chip via biotin-streptavidin interaction. The concentration of crude fraction of protein was 150 μg/ml, the non specific competing *E. coli* DNA was 100 μg/ml and the specific competing oligonucleotide was 10^{-7} M (the repressor R1 has a Kd of 10^{-9} M).

Panels A, B, C show the binding kinetics of the repressor R1 alone (A) or in combination with a second protein factor R2 (C). When tested alone (A & B) both R1 and R2 bind to DNA with different kinetics, with R1 binding more rapidly than R2, whereas when mixed, R1 plus R2 gave a very rapid synergistic binding to the DNA (C). Identical results were obtained by the classical gel mobility shift assay (data not shown). The resulting complex of R1–R2 with DNA can be partially displaced by adding 10^{-7} M of the free DNA template. The sequential addition of R1 and R2 or vice versa (A and B) does not elicit any synergism in the binding of the protein to DNA.

This method presents several advantages over the classical gel mobility shift assay. It does not require any radioactively labeled DNA, more than 1 protein can be tested in a very short time (15–20 minutes) either simultaneously or sequentially and the system shows directly the kinetics of binding as it occurs (real time kinetics). Finally for kinetic studies the system uses far less proteins than the classical gel shift assay. In combination with the gel mobility shift assay, the 'BIA core' system provides a powerful new tool to analyze the interactions of proteins with DNA.

ACKNOWLEDGEMENTS

We would like to thank very much Drs. U. Jönsson and U.W. Roder from Pharmacia for their help to carry out these experiments in their laboratory in Freiburg (Pharmacia, Postfach 5480, D-7800, Freiburg).

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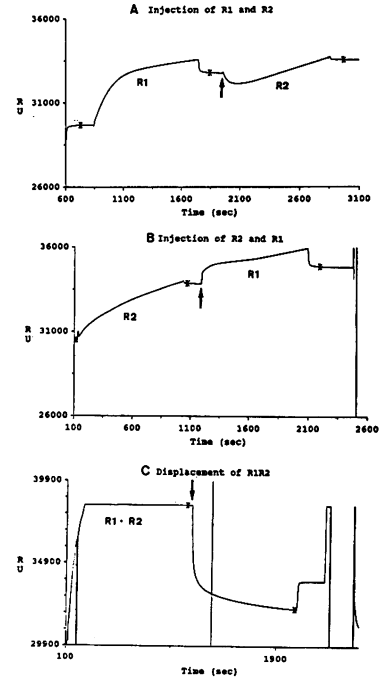

Figure 1: Original diagrams obtained with the Pharmacia BIA core system. The resonance units (RU) are plotted against time of reaction in seconds. The arrows pointing upward in Panels A and B represent the time of injection of the second protein R2 and R1 respectively, whereas the arrow pointing downward represents the time of injection of the free oligonucleotide. R1 is a repressor protein isolated from rooster liver nuclei and R2 is a second protein isolated from the same source (4). Reactions temperature is 25°C and the injected volume 35 μl.