

# Nicking of the tryptophan synthase $\beta_2$ -subunit at Glu-296 prevents the conformational change undergone on binding the $\alpha$ -subunit

Hans-Josef Linkens\*, Lisa Djavadi-Ohanian and Michel E. Goldberg

*Unité de Biochimie Cellulaire, Institut Pasteur, 28, rue du Dr. Roux, 75724 Paris Cedex 15, France*

Received 19 February 1993

Using a monoclonal antibody as conformational probe it has been shown that the weakly active nicked- $\beta_2$  dimer of tryptophan synthase generated by proteolytic cleavage at Glu-296, does not undergo on association with  $\alpha$  subunit a conformational change known to occur in intact  $\beta_2$  subunit. This  $\alpha$  induced conformational change is also prevented in intact  $\beta_2$  by the coenzyme pyridoxal-5'-phosphate when the substrate L-serine is absent

Conformational change; Tryptophan synthase; Monoclonal antibody; Proteolytic cleavage; Ligand binding

## 1. INTRODUCTION

In a recent study Kaufmann et al. [1] have shown that nicking the tryptophan synthase holo  $\beta_2$  subunit from *E. coli* at the C-terminal side of Glu-296 leads to an active species still capable of interacting with  $\alpha$  subunits, while it is known that the limited proteolysis of  $\beta_2$  with trypsin leads to a nicked protein which is inactive and incapable of binding  $\alpha$  subunits [2,3]. As shown in the X-ray structure performed with the largely homologous enzyme from *Salmonella typhimurium* [4] the cleavage sites are located in a region (hinge region) corresponding to a loop without defined secondary structure. Several residues of the interface between the  $\alpha$  and  $\beta$  subunits are carried by the 11 amino acid peptide that is lost during the proteolytic cleavage of  $\beta_2$  by trypsin. This may account for the lack of association between  $\alpha$  and trypsin nicked  $\beta_2$ . The trypsin and endoproteinase Glu-C nicked  $\beta_2$  retain a quasi native structure, remain dimeric and are able to bind the coenzyme pyridoxal-5'-phosphate and the substrate L-serine. However, while binding of pyridoxal-P to intact  $\beta_2$  is cooperative [5] and seems to obey the two-state model of Monod et al. [6], the fixation of the coenzyme to nicked  $\beta_2$  is no more cooperative [1,7]. Tschopp and

Kirschner [7] had concluded that the nicked protein is 'frozen' in a state with low affinity for pyridoxal-P. The results obtained by Chaffotte and Goldberg [8] on the accessibility to water of the tryptophan residues in trypsin nicked  $\beta_2$  and those obtained by Djavadi-Ohanian et al. [9] by probing trypsin nicked  $\beta_2$  with monoclonal antibodies supported this hypothesis. It is also known, from spectroscopic [5,7,10–12] and immunochemical [9] investigations, that the  $\beta_2$  subunit undergoes conformational rearrangements upon binding of the coenzyme pyridoxal-5'-phosphate, the substrate L-serine, or the  $\alpha$  subunit.

In order to gain a better understanding of the mechanism of inactivation of  $\beta_2$  by limited proteolysis, and more specifically of the effect of the proteolysis on the conformational changes of  $\beta_2$ , it was interesting to investigate if the  $\alpha$  induced conformational change observed in native  $\beta_2$  also occurs in Glu-C nicked  $\beta_2$ . To do this, we chose as conformational probe a monoclonal antibody directed against native  $\beta_2$  that was previously obtained and characterized in our laboratory [13,14]. This monoclonal antibody (93-6) was a good candidate because of the following reason.

Though  $\beta_2$  is a symmetrical dimer [4] and therefore should carry two identical binding sites per  $\beta_2$ , only one Fab 93-6 can bind per  $\beta_2$  [9] probably because of steric hindrance as suggested by small-angle X-ray scattering studies [15]. However it was observed that in the  $\alpha\beta_2$  complex, the second binding site for this antibody became accessible [16]. This unmasking of the second epitope on  $\beta_2$  was interpreted as reflecting a major conformational rearrangement that occurs upon binding of  $\alpha$  to  $\beta_2$ .

The present paper reports investigations, carried out with monoclonal antibody 93-6 on the conformational change undergone by apo and holo  $\beta_2$  upon association

*Correspondence address* L. Djavadi-Ohanian, Unité de Biochimie Cellulaire, Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France. Fax: (33) (1) 4061 3043.

*\*Permanent address:* Universität Witten-Herdecke, Stockumer Strasse 10, D-W5810 Witten, Germany.

*Abbreviations*  $\alpha$ ,  $\beta_2$ ,  $\alpha$  or  $\beta_2$  subunit of *Escherichia coli* tryptophan synthase; reduced  $\beta_2$ , NaBH<sub>4</sub> reduced holo  $\beta_2$ ; Ig, immunoglobulin; mAb, monoclonal antibody; SDS, sodium dodecyl sulfate; PEG 6000, polyethyleneglycol with  $M_r = 6,000$ ; Pyridoxal-P, pyridoxal 5'-phosphate; Tryptophan synthase, EC 4.2.1.20.

with  $\alpha$ , either in the absence or in the presence of the substrate L-serine, and on the effects of nicking  $\beta 2$  with endoproteinase Glu-C on this conformational change.

## 2. MATERIALS AND METHODS

### 2.1. Buffers and reagents

The standard buffer was 0.1 M potassium phosphate, pH 7.8, and 2 mM (ethylenedinitrilo)tetraacetic acid. If present, the concentration of pyridoxal-P\* (from Sigma) was 0.1 mM.

### 2.2. Proteins

Preparation of the  $\alpha$  subunit was performed as described previously [17]. Apo  $\beta 2$  was purified as described by Högberg-Raubaud and Goldberg [3]. Holo  $\beta 2$  was obtained by incubation of apo  $\beta 2$  in the presence of 0.1 mM pyridoxal-P.

Nicked  $\beta 2$  was prepared using Endoproteinase Glu-C from *Staphylococcus aureus* V-8 strain, purchased from Boehringer (Mannheim). The preparation and isolation of the single cut nicked  $\beta 2$  was performed as described by Kaufmann et al. [1].

The protein concentrations were determined by measuring the absorbance at 280 nm. The extinction coefficients were 0.6, 0.65, 0.46 and 1.4 for apo  $\beta 2$ , holo  $\beta 2$ ,  $\alpha$  and IgG, respectively [18,19]. Monoclonal antibody mAb 93-6 was prepared as previously described [13,14].

### 2.3. Measurement of the affinity constants

The affinity constant ( $K_A$ ) in solution was determined by the method previously described [20]. Briefly, a constant amount of the antibody was incubated with a varying amount of the antigen, until equilibrium was reached. The quantity of free antibody was monitored by a classical indirect ELISA.

### 2.4. Turbidity measurements

To monitor the precipitation of the mAb 93-6 immune complexes, the turbidity of the mixture was measured in 1 cm cuvettes at 500 nm. At this wavelength no absorbance in the absence of precipitation was observed.

### 2.5. Precipitation of the immune complex with an anti-mouse antibody

The immune complex was formed by incubation of  $\alpha$  and  $\beta 2$  (100  $\mu$ M and 5  $\mu$ M, resp.) for 15 min at room temperature (RT), subsequent addition of the mAb (93-6, 10  $\mu$ M) and incubation for a minimum of 6 h at RT. Then rabbit immunoglobulins anti-mouse Ig (Biosys-France) were added (10  $\mu$ M). After 6 h incubation at RT, precipitation was induced by addition of an equal volume of 7.5% PEG 6000 (Riedel-De Haën, Germany) and incubation for 1 h at 4°C. After centrifugation for 10 min at  $13,000 \times g$  and 4°C, the pellet was rinsed twice with water to remove the remaining PEG 6000 and suspended in sample buffer without  $\beta$ -mercaptoethanol for further SDS gel electrophoresis.

### 2.6. SDS gel electrophoresis

The electrophoresis was performed using the Phast System of Pharmacia (Uppsala, Sweden). The gels used were 8–25% acrylamide gradient Phast gels, the sample buffer did not contain  $\beta$ -mercaptoethanol.

## 3. RESULTS

As reported before, only one Fab of monoclonal antibody 93-6 can bind per  $\beta 2$  dimer [9] and the binding of  $\alpha$  to apo  $\beta 2$  is accompanied by the unmasking of the second antibody binding site on the antigen [16]. Consequently, the binding of two bivalent Ig 93-6 molecules to apo  $\alpha\beta 2$  leads to the formation of a high molecular weight complex and to the precipitation of the antigen–

antibody complex. On the other hand when only one site is accessible, no precipitation occurs. We therefore chose to use the precipitation phenomenon as a measure of the number of binding sites available on  $\beta 2$ . The precipitate was evaluated spectroscopically by measuring the turbidity of the mixture at 500 nm.

### 3.1. The $\alpha$ -induced conformational change analyzed in native $\beta 2$

First, apo  $\beta 2$  (5  $\mu$ M) was incubated for 15 min with  $\alpha$  (20  $\mu$ M) to allow the formation of the  $\alpha\beta 2$  complex. These concentrations of  $\alpha$  and  $\beta 2$  resulted in complete saturation of  $\beta 2$  since the equilibrium dissociation constant of  $\alpha\beta 2$  is about  $2 \cdot 10^{-7}$  M [21]. Then Ig 93-6 (10  $\mu$ M) was added and the turbidity of the solution was measured at different time intervals. As shown in Fig. 1a, a plateau was reached after 1 h. In order to check the nature of the polypeptides present in the precipitate, the mixture containing  $\alpha$ ,  $\beta 2$  and Ig 93-6, incubated together for 1 h, was centrifuged for 10 min at  $5,000 \times g$ . The pellet was dissolved in the sample buffer and analyzed by SDS gel electrophoresis. The pattern obtained after staining with Coomassie blue showed the bands corresponding to the IgG of the antibody and to the  $\alpha$  and  $\beta$  subunits. When Ig 93-6 and apo  $\beta 2$  at the same concentrations as above were mixed in the absence of  $\alpha$ , no precipitation was detected even after 3 h of incubation (data not shown). These observations confirm that, in the presence of  $\alpha$  only, a precipitate containing  $\alpha$ ,  $\beta$  and  $\gamma$  93-6 was formed and that the formation of the precipitate was completed within about 60 min. To investigate the effect of the specific ligands pyridoxal-P and L-serine on the changes undergone by  $\beta 2$  upon

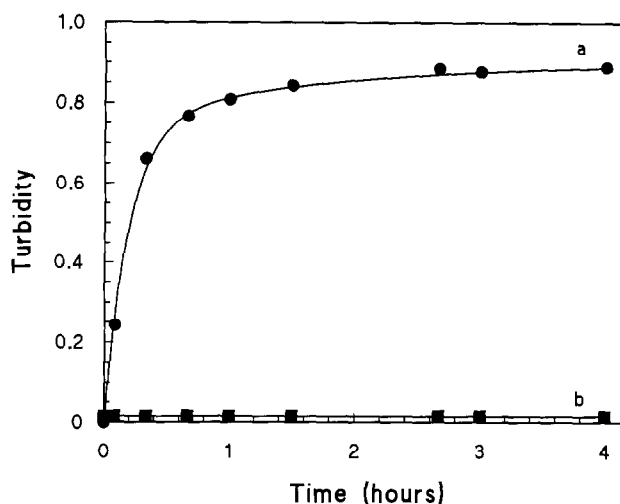


Fig. 1 Precipitation of  $\alpha\beta 2$  monitored after addition of the mAb 93-6 in the absence or presence of pyridoxal-P, and of L-serine.  $\beta 2$  (5  $\mu$ M) was incubated with  $\alpha$  (20  $\mu$ M) and Ig 93-6 (10  $\mu$ M) was added after 15 min. The turbidity of the mixture was measured at 500 nm immediately and at different time intervals (a) apo  $\alpha\beta 2$  or holo  $\alpha\beta 2$  in the presence of 0.1 M L-serine, (b) holo  $\alpha\beta 2$  without serine.

binding of  $\alpha$ , we studied the influence of these ligands on the extent and kinetics of the precipitation.

(a) *Effect of the coenzyme, pyridoxal-P* To analyze the effect of pyridoxal-P on the changes induced by  $\alpha$  on  $\beta_2$ , the precipitation was measured after the addition of Ig 93-6 (10  $\mu$ M) to a mixture of holo  $\beta_2$  (5  $\mu$ M) and  $\alpha$  (20  $\mu$ M). In contrast to the observation made with apo  $\alpha_2\beta_2$ , no precipitation was observed with the holo form of the protein (Fig. 1b). Thus the binding of the coenzyme was shown to prevent the  $\alpha$ -dependent conformational rearrangement of the  $\beta_2$  subunit responsible for the unmasking of the second binding site for monoclonal antibody 93-6.

(b) *Effect of the substrate L-serine* It is known that apo  $\beta_2$  does not bind L-serine and that the binding of L-serine to holo  $\beta_2$  is accompanied by a conformational change in  $\beta_2$  [9,11]. To check whether or not L-serine might also affect the conformation of the holo  $\alpha_2\beta_2$  complex, we probed its effect on the reactivity of holo  $\alpha_2\beta_2$  with the antibody 93-6. Adding Ig 93-6 (10  $\mu$ M) to a mixture of holo  $\beta_2$  (5  $\mu$ M) and  $\alpha$  (20  $\mu$ M) containing 0.1 M L-serine resulted in a precipitation equivalent to that observed in the case of apo  $\alpha_2\beta_2$  (Fig. 1a). Thus, the presence of L-serine released the constraint exerted on  $\beta_2$  by the coenzyme that prevented the precipitation in the absence of serine.

In holo  $\beta_2$  as well as in holo  $\alpha_2\beta_2$ , a Schiff's base is formed between the  $\epsilon$ -amino group of lysine 87 and pyridoxal-P. In the presence of L-serine, this Schiff's base is broken, and a new one is formed between the  $\alpha$ -amino group of the substrate and the coenzyme. The fact that the second binding site for the monoclonal antibody 93-6 is unmasked by the fixation of  $\alpha$  on apo  $\beta_2$  and holo  $\beta_2$  with L-serine, but not on holo  $\beta_2$  in the absence of L-serine, suggested that the presence of the Schiff's base between lysine 87 and pyridoxal 5'-phosphate might prevent the conformational change induced by  $\alpha$ . If this were the case, no precipitation by monoclonal antibody 93-6 of sodium borohydride reduced holo  $\alpha_2\beta_2$  should occur in the presence of L-serine, since the reduction of the Schiff's base renders irreversible the covalent bond between pyridoxal-P and lysine 87. This was checked by adding Ig 93-6 (10  $\mu$ M) to a mixture of reduced holo  $\beta_2$  (5  $\mu$ M) and  $\alpha$  (20  $\mu$ M) containing 0.1 M L-serine. No precipitation was observed, in contrast to the case of non reduced holo  $\alpha_2\beta_2$ , with L-serine (Table I). This suggested that the Schiff's base may play an important role in preventing the  $\alpha$ -induced conformational change in the case of holo  $\beta_2$ .

### 3.2. The $\alpha$ -induced conformational change analyzed in Glu-C nicked $\beta_2$

It was interesting to see if the Glu-C nicked  $\beta_2$  protein, which retains some residual activity and the capacity to bind  $\alpha$  subunit [1], also retains the ability to undergo a conformational change upon binding  $\alpha$ . To investigate this through the immunochemical approach

described above, it was first necessary to determine the affinity constant of monoclonal antibody 93-6 for Glu-C nicked  $\beta_2$  to ensure that the proteolytic cleavage did not result in a drastic modification of the epitope.

The affinity constant in solution was therefore measured using the ELISA method previously described [20]. The affinity constants of monoclonal antibody 93-6 were found to be 2.2, 3 and 4  $\cdot 10^8$  M<sup>-1</sup>, respectively, for the apo, holo and holo with serine forms of Glu-C nicked  $\beta_2$ . These values were not significantly different from one another, and were close to those reported [9] for trypsin nicked apo or holo  $\beta_2$  and for intact holo  $\beta_2$  with or without L-serine ( $K_A = 4.2 \cdot 10^8$  and 5.9  $\cdot 10^8$  M<sup>-1</sup>, respectively).

The fact that the proteolytic cleavage did not affect significantly the binding of monoclonal antibody 93-6 to nicked  $\beta_2$ , allowed us to investigate the eventual change induced by  $\alpha$  on Glu-C nicked  $\beta_2$  with the same precipitation test as that used for intact  $\beta_2$ . However, since the association constant of  $\alpha$  to Glu-C nicked  $\beta_2$  is only about 2  $\cdot 10^{-5}$  M<sup>-1</sup> [1], we used a 100  $\mu$ M concentration of  $\alpha$ , high enough to saturate nicked  $\beta_2$  (5  $\mu$ M).

Neither for nicked apo  $\beta_2$  nor for nicked holo  $\beta_2$  or nicked holo  $\beta_2$  with serine could precipitation be detected after the addition of  $\alpha$  (100  $\mu$ M) and Ig 93-6 (10  $\mu$ M) (Table I) suggesting that the second epitope did not become accessible upon binding of  $\alpha$ . To ascertain this conclusion, it was however important to verify that the ternary complex between  $\alpha$ , nicked  $\beta_2$  and one molecule of Ig 93-6 was indeed formed. To do this, an immunoprecipitation experiment was carried out as described in Section 2 according to the following rationale: nicked  $\beta_2$ ,  $\alpha$  subunit and Ig 93-6 were mixed together, and the immunoglobulin (free or bound to nicked  $\beta_2$  or nicked  $\alpha_2\beta_2$ ) present in the solution was specifically precipitated with a rabbit anti-mouse antibody. The nature of the polypeptide chains present in the immune complex was then analyzed by gel electrophoresis. As control, similar experiments were performed without any  $\beta_2$  or with native  $\beta_2$  instead of nicked  $\beta_2$ .

The gel shown in Fig. 2 revealed that  $\alpha$  was indeed

Table I

Precipitation of  $\beta_2$  with monoclonal antibody 93-6 observed in the absence or presence of the specific ligands or after nicking the protein with endoproteinase Glu-C

| Ligands                | mAb 93-6 with |                   |                   |                              |
|------------------------|---------------|-------------------|-------------------|------------------------------|
|                        | $\beta_2$     | $\alpha_2\beta_2$ | Nicked- $\beta_2$ | $\alpha_2$ Nicked- $\beta_2$ |
| None                   | —             | +                 | —                 | —                            |
| Pyridoxal-P            | —             | —                 | —                 | —                            |
| Pyridoxal-P and serine | —             | +                 | —                 | —                            |

+, presence of precipitation monitored as described in Fig. 1, —, no precipitation.

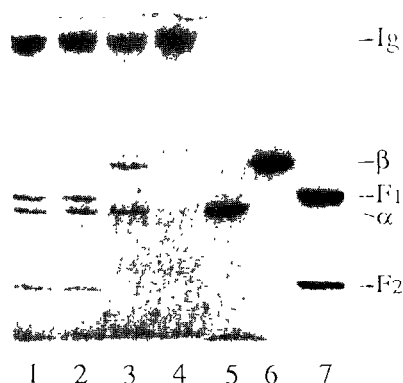


Fig. 2. SDS gel electrophoresis of the immune complexes after precipitation with an anti-mouse antibody. As described in Section 2,  $\beta_2$ ,  $\alpha$ , and the monoclonal antibody were mixed and the immune complex was precipitated with an anti-mouse Ig antibody. The pellet obtained after centrifugation was analyzed by gel electrophoresis in the presence of SDS. Slot 1: nicked holo  $\alpha_2\beta_2$ . Slot 2: nicked holo  $\alpha_2\beta_2$  in the presence of 0.1 M L-serine. Slot 3: holo  $\alpha_2\beta_2$ . Slot 4: without  $\beta_2$ . Slots 5, 6, 7:  $\alpha$ ,  $\beta_2$ , nicked  $\beta_2$  as markers.

present in the immune complex in the experiments with nicked holo  $\beta_2$  without or with serine (slots 1 and 2) as is the case for intact holo  $\beta_2$  (slot 3). The same result (not shown) was obtained with nicked apo  $\beta_2$ . On the other hand no band corresponding to  $\alpha$  could be detected in the experiment where  $\beta_2$  was absent (slot 4) indicating that contamination of the pellet through non-specific binding of  $\alpha$  was negligible. These results clearly showed that a ternary complex containing  $\alpha$ , nicked  $\beta_2$  and Ig 93-6 could be formed, but did not precipitate. This confirmed that the second binding site for monoclonal antibody 93-6 was not unmasked upon association of  $\alpha$  to Glu-C nicked apo  $\beta_2$  or holo  $\beta_2$  plus serine, unlike what happens for native apo  $\beta_2$  and holo  $\beta_2$  plus serine.

#### 4. CONCLUSIONS

It has been previously shown that the *E. coli* tryptophan synthase  $\beta_2$  dimer which should carry two epitopes for each monoclonal antibody, binds only one Fab of mAb 93-6 probably because of steric hindrance [15], while  $\alpha_2\beta_2$  binds two Fab 93-6 [16]. In the quaternary structure of the tetrameric complex of  $\alpha_2\beta_2$  [4] the two  $\beta$  subunits which are at the center of the complex interact over a broad nearly flat surface through which a dyad axis of symmetry passes. The unmasking of the second Fab 93-6 binding site on apo  $\beta_2$  upon binding  $\alpha$  could originate from a change in the relative position of the two  $\beta$  chains that carry the epitopes of the mAb promoting a significant rearrangement in the quaternary structure of  $\beta_2$ .

The results reported in this paper first show that the binding of the coenzyme pyridoxal-P and of the substrate L-serine has an important influence on the  $\alpha$ -

induced conformational rearrangement of the  $\beta_2$  subunit. Holo  $\beta_2$  in contrast to apo  $\beta_2$ , is unable to undergo the change leading to the unmasking of the second binding site for the antibody 93-6. However, holo  $\beta_2$  in the presence of L-serine behaves as apo  $\beta_2$  and can bind two Fab 93-6 when associated to  $\alpha$ . Thus L-serine releases the constraint imposed to  $\beta_2$  by the coenzyme. The finding that L-serine does not release this constraint in reduced-holo  $\beta_2$  suggests that the Schiff's base between the coenzyme and the protein plays a role in controlling the balance between the two conformational states of  $\beta_2$ . During the catalytic cycle of tryptophan synthase, the enzyme constantly turns over from a state with the Schiff's base with Lys-87 and no bound serine, to a state with bound serine and no Schiff's base between the coenzyme and Lys-87. It therefore seems very likely that the  $\beta_2$  subunit keeps oscillating between the two conformational states identified with mAb 93-6.

As reported by Kaufmann et al. [1], the proteolytic cleavage of holo  $\beta_2$  subunit at Glu-296 leads to a partly active species capable of weakly interacting with  $\alpha$  subunits (Kaufmann et al., 1991).  $\beta_2$  cleaved at other positions in the hinge region (Lys-272, Arg-275, Lys-283) is enzymatically inactive and cannot associate with the  $\alpha$  subunit [2,3].

We have shown here that neither for Glu-C nicked apo  $\beta_2$  nor for nicked holo  $\beta_2$  or nicked holo  $\beta_2$  with L-serine, does the second binding site of mAb 93-6 become readily accessible after the association of  $\alpha$ , indicating that the single cut at Glu-296 prevents the protein from undergoing the conformational rearrangement observed with the intact  $\beta_2$  upon the association of  $\alpha$ . That nicked  $\beta_2$  which has a reduced enzymatic activity, fails to readily undergo the  $\alpha$ -induced conformational rearrangement identified with monoclonal antibody 93-6, suggests that this rearrangement may be an essential part of the catalytic mechanism of the  $\alpha_2\beta_2$  complex.

**Acknowledgements:** The authors thank Pr. Peter Bartholmes for helpful discussions and his interest in this work. This work was supported by funds from the Institut Pasteur, the Centre National de la Recherche Scientifique (CNRS URA 1129) and the Université Paris 7. One of the authors (H.J.L.) was supported by a grant from the Deutsche Forschungsgemeinschaft (Ba 527/6-1) and a Deutscher Akademischer Austauschdienst short-term Fellowship.

#### REFERENCES

- [1] Kaufmann, M., Schwarz, T., Jaenicke, R., Schnackerz, K.D., Meyer, H.E. and Bartholmes, P. (1991) *Biochemistry* 30, 4173-4179.
- [2] Högberg-Raibaud, A. and Goldberg, M.E. (1977) *Proc. Natl. Acad. Sci. USA* 74, 442-446.
- [3] Högberg-Raibaud, A. and Goldberg, M.E. (1977) *Biochemistry* 16, 4014-4020.
- [4] Hyde, C.C., Ahmed, S.A., Padlan, E.A., Miles, E.W. and Davies, D.R. (1988) *J. Biol. Chem.* 263, 17857-17871.
- [5] Bartholmes, P., Kirschner, K. and Gschwind, H.-P. (1976) *Biochemistry* 15, 4712-4717.

- [6] Monod, J., Wyman, J. and Changeux, J.-P. (1965) *J. Mol. Biol.* 12, 88–118.
- [7] Tschopp, J. and Kirschner, K. (1980) *Biochemistry* 19, 4514–4521.
- [8] Chaffotte, A.F. and Goldberg, M.E. (1984) *Eur. J. Biochem.* 139, 47–50.
- [9] Djavadi-Ohanian, L., Friguet, B. and Goldberg, M.E. (1986) *Biochemistry* 25, 2502–2508.
- [10] Bartholmes, P. and Teuscher, B. (1979) *Eur. J. Biochem.* 95, 323–326.
- [11] Lane, A.N. and Kirschner, K. (1983) *Eur. J. Biochem.* 129, 561–570.
- [12] Lane, A.N., Paul, C.H. and Kirscher, K. (1984) *EMBO J.* 3, 279–287.
- [13] Djavadi-Ohanian, L., Friguet, B. and Goldberg, M.E. (1984) *Biochemistry* 23, 97–104.
- [14] Friguet, B., Djavadi-Ohanian, L. and Goldberg, M.E. (1984) *Mol. Immunol.* 21, 673–677.
- [15] Wilhelm, P., Friguet, B., Djavadi-Ohanian, L., Pilz, I. and Goldberg, M.E. (1987) *Eur. J. Biochem.* 164, 103–109.
- [16] Murry-Brelier, A. and Goldberg, M.E. (1990) *J. Biol. Chem.* 265, 7987–7993.
- [17] Hatanaka, M., White, E.A., Horibata, K. and Crawford, I.P. (1962) *Arch. Biochem. Biophys.* 97, 596–606.
- [18] Miles, E.W. (1970) *J. Biol. Chem.* 215, 6016–6025.
- [19] Onoue, K., Yagi, Y., Grossberg, A.L. and Pressman, D. (1965) *Immunochemistry* 2, 401–415.
- [20] Friguet, B., Chaffotte, A.F., Djavadi-Ohanian, L. and Gold-