Immunology 1989 **67** 148–153

Monoclonal antibodies to human growth hormone induce an allosteric conformational change in the antigen

M. M. MAZZA & L. A. RETEGUI *Instituto de Quimica y Fisicoquimica Biológicas (UBA-CONICET), Facultad de Farmacia y Bioquimica, Junin, 956–1113 Buenos Aires, Argentina*

Accepted for publication 31 January 1989

SUMMARY

We re-investigated the properties of a monoclonal antibody (mAb), 4D11, to human growth hormone (hGH) that showed a very weak affinity, recognizing hGH only when the hormone was solubilized on a solid surface. MAb 4D11 did not significantly bind ^{125}I-hGH. It was found that three mAb directed to different hGH epitopes (mAb 3C11, 10C1 and NA71) were able to induce the binding of the soluble antigen to mAb 4D11. The co-operative effect could be demonstrated by the formation of binary complexes (Ag:Ab, 1:2) detected by high-performance liquid chromatography (HPLC) and by the increase of radioactivity found when the synergistic mAb were added to ^{125}I-hGH incubated with mAb 4D11 immobilized on polyvinyl microplates. Other possible explanations, such as the formation of cyclic complexes or the generation of a new epitope in the Fc fragment of the first antibody (Ab), were dismissed because the Fab fragment of one of the enhancing mAb (3C11) gave the same effect as the intact Ab. The data suggest that the hGH molecule undergoes a localized conformational change after binding to mAb 3C11, NA71 or 10C1 and that mAb 4D11 binds with high affinity to the modified region of the hormone. The formation or not of ternary complexes (Ag:Ab, 1:3) was used to localize the 4D11 epitope on the surface of the Ag. It is suggested that mAb 4D11 recognizes a conformational change produced in the region defined by the AE5/AC8 epitopes, which is close to the hGH antigenic domain only expressed when the protein is immobilized on plastic surfaces.

INTRODUCTION

The relatively frequent finding that the binding of one monoclonal antibody (mAb) to a soluble antigen (Ag) increases the binding of a second antibody (Ab) has been explained by different mechanisms. Moyle, Anderson & Ehrlich (1983) and Holmes & Parham (1983) have described the formation of highly stable cyclic complexes of Ag and Ab against different antigenic sites, and Nemazee & Sato (1982) have hypothesized that the second Ab would recognize an epitope expressed on the first Ab as a consequence of its binding to the Ag. A third possibility, which implies that the enhancing Ab produces an allosteric change in the Ag that modifies the epitope recognized by the second Ab, has been proposed by Diamond, Butcher & Howard (1984) studying mAb to rat class I major transplantation Ag, a polymorphic transmembrane molecule. Induced conformational changes in soluble, highly purifed Ag, have not yet been reported. On the contrary, crystallographic data of the complex formed by the Fab fragment of a mAb to egg white lysozyme and the Ag showed no evidence of conformational alterations in the lysozyme molecule (Amit *et al.*, 1986).

Correspondence: Dr L. A. Retegui, Instituto de Quimica y Fisicoquimica Biológicas, Facultad de Farmacia y Bioquimica, Junin 956, 1113 Buenos Aires, Argentina.

During the study of the antigenic topography of human growth hormone (hGH) (Vita *et al.*, 1986; Mazza & Retegui, 1989), we found a mAb (mAb 4D11) which bound hGH adsorbed on plastic surfaces with low affinity but did not bind soluble hGH at all. However, each one of three different mAb to hGH induced the binding of hGH to mAb 4D11. Since the Fab fragment of one of the enhancing Ab retained the ability to produce the co-operative effect, we suggest that an allosteric conformational change has occurred in the Ag which leads to mAb 4D11 binding. The tentative localization of the region recognized by mAb 4D11 on the antigenic surface of hGH is proposed.

MATERIALS AND METHODS

Human growth hormone

hGH was prepared in our laboratory following the method of Mills *et al.* (1969) and labelled with ^{125}I as described by Roth (1975). Specific radioactivities of 70–140 μCi/μg were usually achieved.

Monoclonal antibodies

MAb anti-hGH QA68, NA39, NA71, NA27 and anti-human placental lactogen (hPL) EB1, EB2 and EB3 were kindly

provided by Dr J. Ivanyi (Ivanyi & Davies, 1980; Ivanyi, 1982). MAb anti-hGH 10D5, 10D6, 3C11, 10C1, 4D11, 04C11, F11, AE12, AE5, AC3 and HG3 have been prepared and characterized by the authors (Retegui *et al.*, 1982; Retegui, Masson & Paladini, 1985; Mazza & Retegui, 1989).

MAb 3C11 was purified from ascitic fluid by affinity chromatography on a protein A–Sepharose 4B column (Ey, Prowse & Jenkin, 1978). The pH 6·0 fraction was dialysed against 0·1 M phosphate, pH 7·4, concentrated and stored at −20°. The Fab fragment was obtained by digestion of the mAb (0·2 mg/ml) with 1% (w/w) papain (Sigma Chemical Co., St Louis, MO) in 75 mM phosphate buffer, pH 7·0, containing 10 mM NaCl, 20 mM EDTA and 10 mM cysteine. After 18 hr at 37°, the reaction was stopped by the addition of iodoacetamide (final concentration 25 mM).

High-performance liquid chromatography (HPLC) of immune complexes

The size of the immune complexes formed by ^{125}I-hGH and mAb was determined by gel-filtration HPLC on either a Bio-Sil TSK-250 or Bio-Sil TSK-400 column (Bio-Rad Laboratories, Richmond, CA). The experimental procedure was as follows: approximately 3×10^6 c.p.m. of ^{125}I-hGH and 1 μl of ascitic fluid were incubated 2 hr at 37° and 16 hr at 4° in a total volume of 50 μl of buffer NaH_2PO_4 20 mM, Na_2SO_4 50 mM, pH 6·8, containing 1 mg/ml of bovine serum albumin (BSA). After clarification in an Eppendorf microcentrifuge, the samples were diluted 30 times with the same buffer and 20 μl of the dilutions injected into the HPLC column. Elution was carried out at a flow rate of 1 ml/min with the buffer cited before, without BSA. Fractions of 230–250 μl were collected. The radioactivity present in the effluent was measured in a gamma counter. In some experiments the effluent was monitored at 280 nm. The columns were calibrated with the reference proteins: thyroglobulin, IgG, ovalbumin, myoglobin and cyanocobalamin (Bio-Rad MW gel filtration standard) and with ^{125}I-hGH and immune complexes of known size prepared with this Ag (Mazza & Retegui, 1989).

Radioimmunoassays (RIA)

The effect of various mAb, or their fragments, on the ^{125}I-hGH binding to immobilized mAb 4D11 was studied using a solid-phase RIA, as described previously (Etcheverrigaray, Paladini & Retegui, 1988). Briefly, polyvinyl microplates coated with mAb 4D11 (ascitic fluid diluted 1:500 in 5 mM glycine buffer, pH 9·2) were incubated with ^{125}I-hGH (final concentration 4·5–9·0 ng/ml) and serial dilutions of either a mAb or its fragments. Both labelled hGH and competitor were diluted in phosphate-buffered saline (PBS) containing 10 mg/ml of BSA. After incubating for 4 hr at 37° and overnight at room temperature, the plates were washed with PBS containing 0·125 ml Tween 20/litre and the bound radioactivity counted.

Liquid-phase RIA were performed as described by Peña, Poskus & Paladini (1980).

RESULTS

Inductive effect of various mAb anti-hGH on the binding of ^{125}I-hGH to mAb 4D11

When mAb 4D11 was prepared it was characterized as a mAb of very weak affinity, recognizing hGH only when the hormone was immobilized on polypropylene microplates or on latex particles (Retegui *et al.*, 1982) The binding of ^{125}I-hGH to mAb 4D11 was so low (20–30 times less than other mAb obtained at the same time) that it could not be used in competition experiments. Reinvestigating the properties of mAb 4D11 we found that some mAb anti-hGH were capable of inducing its binding to ^{125}I-hGH. Figure 1 shows that when a mixture of the tracer and mAb 4D11 was incubated and then submitted to HPLC fractionation, the only radioactive material eluted corresponded to ^{125}I-hGH (22,000 MW), indicating the absence of binding to the mAb. However, the addition of mAb 3C11 to the incubation mixture produced a radioactive material which eluted with a MW of 300,000, corresponding to the formation of 1:2 Ag:Ab complexes. The formation of such a binary complex suggests the simultaneous binding of both mAb 3C11 and 4D11 to ^{125}I-hGH. MAb EB3 was unable to induce the binding of mAb 4D11 to the tracer since the pattern of radioactivity shown in Fig. 1 indicates the formation of a unitary Ag–Ab complex (Ag:Ab, 1:1 ratio, 150,000 MW). When a set of 11 mAb anti-hGH (namely mAb F11, AE12, 3C11, NA71, HG3, 10C1, 10D6, EB1, EB3, AC8 and 04C11) were tested as described above, only three, i.e. mAb 3C11, NA71 and 10C1, produced binary complexes in the presence of mAb 4D11, indicating the simultaneous binding of both mAb (data not shown).

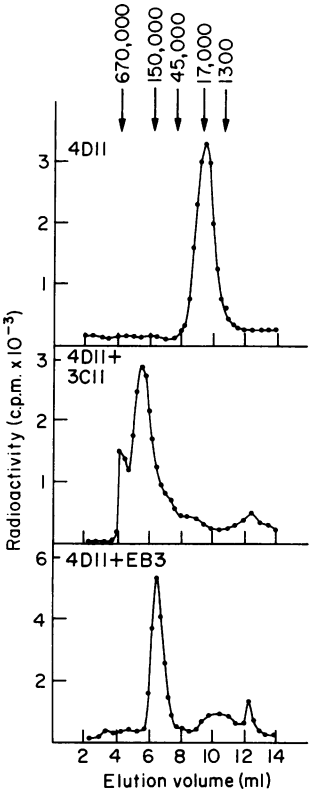

Figure 1. Effect of mAb 3C11 or mAb EB3 on the ^{125}I-hGH binding to mAb 4D11. The tracer (3×10^6 c.p.m.) was incubated with the corresponding mAb (ascitic fluid diluted 1:50) and the mixture submitted to HPLC on a Bio-Sil TSK-250 column as described in the Materials and Methods. Numbers at the top indicate the MW of the markers used to calibrate the column.

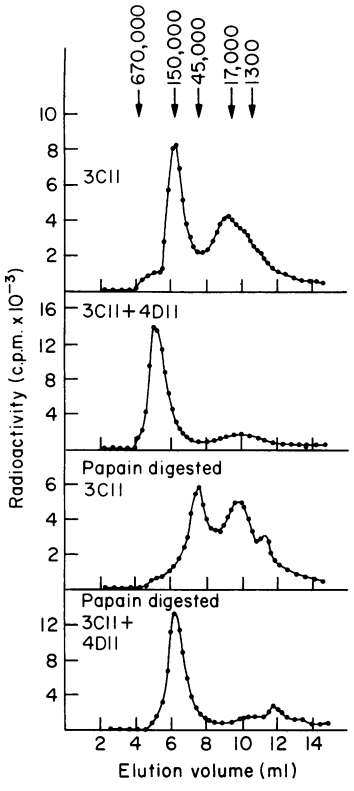

Figure 2. Inductive effect of purified mAb 3C11 and its papain digest on the ^{125}I-hGH binding to mAb 4D11. The tracer (3×10^6 c.p.m.) was incubated in a final volume of 50 μl with purified mAb 3C11 (5 μg) or its papain digest (3·5 μg). When indicated, mAb 4D11 (ascitic fluid 1:50) was added simultaneously. Each mixture was submitted to HPLC on a Bio-Sil TSK-250 column, as described in the Materials and Methods and Fig. 1.

A direct measurement of the Ka value for mAb 4D11 binding to hGH was not possible because of the fact that mAb 4D11 did not significantly bind ^{125}I-hGH. Therefore we compared the ability of hGH to inhibit the binding of ^{125}I-hGH to mAb 3C11 and to a mixture of mAb 3C11 and mAb 4D11. Scatchard analysis of the data indicated a great increase in affinity for the mixture of mAb 3C11 and 4D11, the Ka being $3{\cdot}5 \times 10^9$ M^{-1} in this case versus $4{\cdot}8 \times 10^7$ M^{-1} for mAb 3C11 alone (not shown).

A similar co-operative effect of some mAb on the behaviour of mAb 4D11 was found in a solid-phase RIA. Each mAb (ascitic fluid diluted 1:1000) and ^{125}I-hGH (80,000 c.p.m./well) were added to polyvinyl microplates coated with mAb 4D11. Whereas insolubilized mAb 4D11 bound approximately 1% of the added radioactivity, the addition of mAb 3C11 or 10C1 increased this value to 47% and 32%, respectively. MAb F11, QA68, AE12, AE5, AC3, 10D5, NA27, NA71, HG3, 10D6, EB1, EB3, AC8, 04C11 and EB2 did not show any effect.

Inductive effect of Fab fragment from mAb 3C11 on the binding of ^{125}I-hGH to mAb 4D11

Figure 2 shows that purified mAb 3C11 also induces the binding of ^{125}I-hGH to mAb 4D11, discarding any artefactual effect of non-antibody constituents of the ascitic fluid.

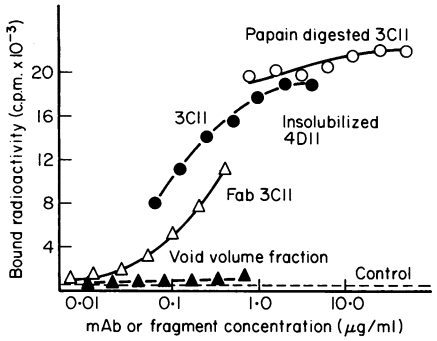

Figure 3. Effect of purified mAb 3C11, its papain digest or the Fab fragment isolated from it on the ^{125}I-hGH binding to insolubilized mAb 4D11. The tracer (88,000 c.p.m./well) was incubated with the different concentrations of competitors in polyvinyl microplates coated with mAb 4D11, as described in the Materials and Methods. The void volume fraction corresponds to the material of MW $\geqslant$ 670,000 isolated from the papain digest of mAb 3C11. Control means the radioactivity bound in the absence of any competitor (660 c.p.m.).

To study the influence of the Ab structure on co-operativity, mAb 3C11 was digested with papain to prepare the Fab fragment. The whole digest was incubated with ^{125}I-hGH and the mixture submitted to HPLC fractionation. The analysis of the pattern of radioactivity obtained showed the formation of a complex with 45,000 MW instead of 150,000 MW, indicating the absence of intact immunoglobulin. A similar assay was carried out incubating the papain-digested mAb 3C11 with the tracer and mAb 4D11. The formation of a complex of MW close to 150,000 and the absence of free ^{125}I-hGH indicated that the co-operative effect was still present (Fig. 2).

In order to purify the Fab fragment the mAb 3C11 digest was submitted to HPLC on a Bio-Sil TSK-250 column as described in the Materials and Methods. Fractions corresponding to the void volume (4·0–4·5 ml), which included material of 670,000 MW and those which include the Fab fragment (45,000 MW, 7·3–8·0 ml), were pooled and subsequently assayed in a solid-phase RIA (Fig. 3). The results demonstrated that both the whole 3C11 digest and the Fab fragment retained the ability of inducing the binding of ^{125}I-hGH to insolubilized mAb 4D11. The material eluting in the void volume of the column was ineffective in the same assay and its presence was not detected in the experiments described in Fig. 2, suggesting that it was devoid of Ab activity. Moreover, as this material was also detected when mAb 3C11 purified through a column of Sepharose–protein A was submitted to HPLC, it could probably be due to some protein co-eluting with the mAb during the affinity chromatography.

Localization of the 4D11 epitope on the hGH antigenic map

Mapping studies based on the ability of paired mAb to bind simultaneously to the Ag are possible when each mAb tested alone binds the tracer with a similar affinity. The formation of unitary or binary complexes suggests that the epitopes recognized are overlapping or independent, respectively. The results obtained with this group of antibodies must exhibit a coherence that allows anomalous reactions of mAb to be dismissed (Vita *et al.*, 1986; Mazza & Retegui, 1989).

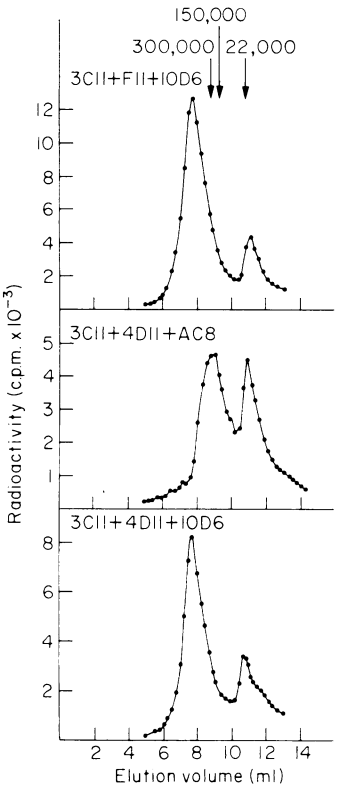

Figure 4. Ability of mixtures of three mAb to bind simultaneously or not to ^{125}I-hGH. The tracer (3×10^6 c.p.m.) was incubated with the corresponding mAb (ascitic fluid diluted 1:50) and the mixture submitted to HPLC fractionation on a Bio-Sil TSK-400 column, as described in the Materials and Methods. Numbers at the top indicate the MW of the markers used to calibrate the column.

Owing to the fact that mAb 4D11 bound ^{125}I-hGH only after the previous binding of some other mAb, the formation of an unitary complex when mAb 4D11 and a second one were incubated simultaneously with the Ag would indicate either the close proximity of both epitopes or the lack of inductive effect of the second mAb. Therefore, to localize the region of the hGH molecule recognized by mAb 4D11 under the influence of a second mAb we explored the formation of ternary complexes. Results in Fig. 4 demonstrate that three mAb can bind simultaneously to ^{125}I-hGH, as is the case for mAb 3C11, F11 and 10D6. The formation of ternary complexes obtained by incubating mAb 3C11 and 4D11 with the tracer in the presence of mAb 10D6 indicates that the epitopes 4D11 and 10D6 must be located so far apart that the simultaneous binding of the mAb is possible. Conversely, the binary complexes detected after the incubation of mAb 3C11, 4D11 and AC8 suggest that the region of the hormone recognized by mAb 4D11 must be close to epitope AC8 (Fig. 4).

Table 1 summarizes the results obtained using various combinations of mAb, whose respective epitopes are represented in Fig. 5, a schematic drawing showing the relative distribution of the hGH antigenic determinants previously established by Mazza & Retegui (1989). Ternary complexes originated by mAb 3C11, 4D11 and a third mAb were detected for mAb F11, 10D6 and 04C11. Mab AE5, AC3, NA71, EB3 and AC8 only gave rise to binary complexes (Table 1).

Table 1. Ability of pairs of mAb to bind simultaneously or not with mAb 4D11 to ^{125}I-hGH

Additions to the incubation mixture*	Immune complex generated	
	Binary†	Ternary‡
3C11+F11	−	+
3C11+AE5	+	−
3C11+AC3	+	−
3C11+NA71	+	−
3C11+10D6	−	+
3C11+EB3	+	−
3C11+AC8	+	−
3C11+04C11	−	+

* ^{125}I-hGH ($\approx 3 \times 10^6$ c.p.m.) was incubated with mAb 4D11 and the indicated mAb, all diluted 1:50 as described in the Materials and Methods.

† Binary means that the MW of the immune complex formed was about 300,000, indicating that only two mAb could bind simultaneously to ^{125}I-hGH.

‡ Ternary means that the MW of the immune complex formed was about 500,000, indicating that the three mAb are bound simultaneously to ^{125}I-hGH.

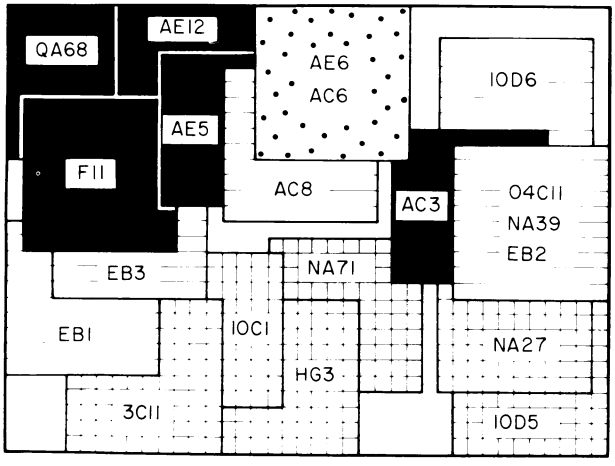

Figure 5. Schematic drawing showing the relative distribution of various hGH epitopes. The rectangle represents the hGH molecular area (7·238 Å^2) and the squares correspond to the area of the epitope recognized by each mAb (690 Å^2). The two-dimensional design retains the essential features of the spatial distribution of the hGH epitopes established previously (Mazza & Retegui, 1989), with the restrictions imposed by transferring a three-dimensional model to a plane. The different symbols indicate the mAb bound in each epitope as follows: solid, mAb with unique specificity for hGH; cross-hatched, mAb cross-reacting with hPL; horizontally stripped, mAb with identical affinity for hGH or hPL. Spotted squares represent the epitopes detected in hGH only after its adsorption on a plastic surface, as detailed elsewhere (Vita *et al.*, 1986; Mazza & Retegui, 1989).

These results support the localization of 4D11 epitope on the hGH antigenic map based on the following reasoning: (i) epitope 4D11 cannot be overlapping with epitopes 3C11, 10C1 and NA71 as the corresponding mAb and mAb 4D11 bound simultaneously to ^{125}I-hGH; (ii) for the same reason epitope 4D11 would not be located in the regions covered by mAb EB3 or mAb AC3, as these epitopes are superimposed with epitopes 10C1 and NA71, respectively (Fig. 5); (iii) the localization in the F11, 10D6 or 04C11 sites is discarded because the formation of ternary complexes is possible (Table 1). Hence all the available evidence points to the hGH antigenic domain close to epitopes AE5 and AC8 (Fig. 5) as the region where mAb 4D11 binds.

DISCUSSION

The results presented in this report show that three mAb directed to different hGH epitopes (mAb 3C11, 10C1 and NA71) were able to induce the binding of the soluble Ag to mAb 4D11. The enhancement factor, i.e. the Ka for a mixture of mAb 3C11 and 4D11 divided by the Ka for mAb 3C11, was about 100. This value is 10 times higher than those reported in the literature for co-operative mAb to human chorionic gonadotropin (Ehrlich *et al.*, 1982).

The Fab fragment from mAb 3C11 was as effective as the intact mAb 3C11 in enhancing the binding of mAb 4D11 to ^{125}I-hGH. This fact eliminates two possible mechanisms to explain the effect: (i) the formation of a cyclic complex because this model requires bivalency of the Ab (Moyle *et al.*, 1983; Holmes & Parham, 1983); and (ii) the recognition by mAb 4D11 of a determinant on the Fc fragment of the enhancing mAb, as proposed by Nemazee & Sato (1982) for their system. It seems unlikely that the co-operative effects shown here could be explained in terms of idiotypic phenomena (the second Ab recognizing a composite site between the first Ab and the Ag) because three different mAb were equally effective. Therefore the data strongly suggest that the hGH molecule undergoes a localized conformational change as the result of its binding to either mAb 3C11, 10C1 or NA71 and that mAb 4D11 recognizes with high affinity the modified region of the hormone.

The knowledge of the relative distribution of hGH epitopes defined by a set of 20 mAb already established by Mazza & Retegui (1989) allowed the localization of the 4D11 epitope on the hormone surface. The information was obtained studying the feasibility of ternary complex formation (Table 1). The data obtained suggest that epitope 4D11 shares the region of the hormone defined by epitopes AE5 and AC8 (Fig. 5). It is interesting to point out that in this area are partially located epitopes AE6 and AC6, which are only expressed when hGH is immobilized on plastic surfaces (Mazza & Retegui, 1989). Hence, these results suggest that the hGH domain recognized by mAb AE5, AC8, AE6, AC6 and 4D11 is easily modified by physical processes, like adsorption to plastic surface or by the binding of certain Ab.

Some results show an apparent lack of agreement. While mAb 3C11, 10C1 and NA71 enhanced the binding of ^{125}I-hGH to mAb 4D11 in liquid phase, only mAb 3C11 and 10C1 produced this effect on immobilized mAb 4D11. The failure of mAb NA71 to enhance the binding of the tracer in solid phase is probably related to the proximity of NA71 and 4D11 epitopes on the hGH surface (Fig. 5). These determinants would be closer neighbours than the epitope pairs 3C11/4D11 and 10C1/4D11, thus originating steric hindrance phenomena which may impair the binding of the complex ^{125}I-hGH:mAb NA71 to the insolubilized mAb 4D11.

It has been suggested that the apparent relationship between the exposed area of a protein and antigenic reactivity may actually reflect the increased mobilities of turns, loops and other highly exposed areas (Tainer *et al.*, 1984). Furthermore, preliminary data on the three-dimensional structure of hGH (Borisova *et al.*, 1988) indicate that this molecule is quite similar to that of pGH established by Abdel-Meguid *et al.* (1987). It seems that a high proportion ($\approx$44%) of loops, turns and near random coil areas, as well as the absence of β-structures, is a common feature within the closely related family of growth hormone. These highly mobile structures would support the probability of occurrence of allosteric conformational changes in hGH under the influence of Ab binding.

As already mentioned, Diamond *et al.* (1984) were able to demonstrate localized modifications induced in the Ag by the binding of a mAb, and other investigators have suggested the occurrence of a similar mechanism to explain their findings (Duncan, Hewitt & Weston, 1982; Parham, 1984; Milne *et al.*, 1987). The results presented in this report suggest that some mAb can produce such an alteration in the hGH molecule. However, the X-ray diffraction study of the three-dimensional structure of a complex formed between lysozyme and a Fab fragment from a mAb did not detect any conformational change in the native enzyme (Amit *et al.*, 1986; Harper *et al.*, 1987). Whether this result is related to a more rigid conformation of the lysozyme molecule compared to that of hGH and/or to the characteristics of the mAb used is not known. The lack of inductive effect of one mAb does not eliminate the possibility that another mAb recognizing a different antigenic site in the same molecule might induce a conformational change.

We have shown that enhancing Ab are present in sera from hypopituitary patients under hGH therapy (Retegui *et al.*, 1985) and in hGH-hyperimmunized mice and hamsters (Etcheverrigaray *et al.*, 1988). Although we did not investigate the mechanism involved we could establish that the generation of enhancing Ab was linked to individual characteristics, either in outbred or inbred populations. We also demonstrated that some mice elicited Ab enhancing the ^{125}I-hGH binding to immobilized mAb 4D11 (Etcheverrigaray *et al.*, 1988).

The use of mAb has made it possible to dissect the wide spectrum of specificities that might comprise an immune response. Thus the antigenicity of circulating Ag–Ab complexes as well as the *in vivo* processing of the Ag may generate enhancing Ab, as described in this report, heteroclitic Ab (Retegui & Paladini, 1986; Etcherverrigaray *et al.*, 1988) and even Ab to non-native Ag (Friguet, Djavadi-Ohaniance & Goldberg, 1984). Whatever their mechanism of action, these Ab represent normal components of conventional antisera, being partially responsible for their affinity and specificity properties.

ACKNOWLEDGMENTS

We are particularly indebted to Professor Alejandro C. Paladini for helpful discussions and critical revision of the manuscript.

The technical assistance of Miss M. Ramírez in the preparation of the manuscript is gratefully acknowledged.

This work was partially supported by grants from the Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET) and the Organization of American States. L. A. Retegui is Career Investigator from CONICET and M. M. Mazza is a fellow from the same institution.

REFERENCES

ABDEL-MEGUID S.S., SHIEH H.-S., SMITH W.W., DAYRINGER H.E., VIOLAND B.N. & BENTLE L.A. (1987) Three-dimensional structure of a genetically engineered variant of porcine growth hormone. *Proc. natl. Acad. Sci. U.S.A.* **84,** 6434.

AMIT A.G., MARIUZZA R.A., PHILLIPS S.E.V. & POLJAK R.J. (1986) Three dimensional structure of antigen-antibody complex at 2·8 Å resolution. *Science*, **233,** 747.

BORISOVA S.N., PAVLOVSKY A.G., VAINSHTEIN B.K., RUBTSOV P.M., SKRYABIN K.G. & BAYEV A.A. (1988) Structure of human growth hormone. *14th International Congress of Biochemistry*. Prague, Czechoslovakia. Abstract FR:072.

DIAMOND A.G., BUTCHER G.W. & HOWARD J.C. (1984) Localized confirmational changes induced in a class I major histocompatibility antigen by the binding of monoclonal antibodies. *J. Immunol.* **132,** 1169.

DUNCAN R.J.S., HEWITT J. & WESTON P.D. (1982) Activation of β-galactosidase by monoclonal antibodies. *Biochem. J.* **205,** 219.

EHRLICH P.H., MOYLE W.R., MOUSTAFA Z.A. & CANFIELD R.E. (1982) Mixing two monoclonal antibodies yields enhanced affinity for antigen. *J. Immunol.* **128,** 2709.

ETCHEVERRÍGARAY M., PALADINI A.C. & RETEGUI L.A. (1988) Stochastic humoral expression of human growth hormone epitopes. *Immunology*, **63,** 595.

EY P.L., PROWSE S.J & JENKIN C.R. (1978) Isolation of pure IgG2a and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. *Biochemistry*, **15,** 429.

FRIGUET B., DJAVADI-OHANIANCE L. & GOLDBERG M.E. (1984) Some monoclonal antibodies raised with a native protein bind preferentially to the denatured antigen. *Molec. Immunol.* **21,** 673.

HARPER M., LEMA F., BOULOT G. & POLJAK R. (1987) Antigen specificity and cross-reactivity of monoclonal antilysozyme antibodies. *Molec. Immunol.* **24,** 97.

HOLMES, N.J. & PARHAM P. (1983) Enhancement of monoclonal antibodies against HLA-A2 is due to antibody bivalency. *J. Biol. Chem.* **258,** 1580.

IVANYI J. (1982) Analysis of monoclonal antibodies to human growth hormone and related proteins. In: *Monoclonal Hybridoma Antibodies: Techniques and Applications* (ed. J. G. R. Hurrell), pp. 59–79. CRC Press, Cleveland.

IVANYI J. & DAVIES P. (1980) Monoclonal antibodies against human growth hormone. *Molec. Immunol.* **17,** 287.

MAZZA M.M. & RETEGUI L.A. (1989) The antigenic topography of human growth hormone. *Molec. Immunol.* **26,** 231.

MILLS J.B., ASHWORTH R.B., WILHELMI A.E. & HARTREE A.S. (1969) Improved method for the extraction and purification of human growth hormone. *J. clin. Endocr. Metab.* **29,** 1456.

MILNE R.W., BLANCHETTE L., THÉOLIS JR. R., WEECH P.K. & MARCEL Y.L. (1987) Monoclonal antibodies distinguish between lipid-dependent and reversible conformational states of human apolipoprotein B. *Molec. Immunol.* **24,** 435.

MOYLE W.R., ANDERSON D.M. & EHRLICH P.H. (1983) A circular antibody-antigen complex is responsible for increased affinity shown by mixtures of monoclonal antibodies to human chorionic gonadotropin. *J. Immunol.* **131,** 1900.

NEMAZEE D.A. & SATO V.L. (1982) Enhancing antibody: a novel component of the immune response. *Proc. natl. Acad. Sci. U.S.A.* **79,** 3828.

PARHAM P. (1984) Changes in conformation with loss of alloantigenic determinants of a histocompatibility antigen (HLA-B7) induced by monoclonal antibodies. *J. Immunol.* **132,** 2975.

PEÑA C., POSKUS E. & PALADINI A.C. (1980) A relevant antigenic site in human growth hormone localized in sequence 98–128. *Molec. Immunol.* **17,** 1487.

RETEGUI L.A., MASSON P.L. & PALADINI A.C. (1985) Specificities of antibodies to human growth hormone (hGH) in patients treated with hGH: longitudinal study and comparison with the specificities of animal antisera. *J. clin. Endocrinol. Metab.* **60,** 184.

RETEGUI L.A., MILNE R.W., CAMBIASO C.L. & MASSON P.L. (1982) The recognition by monoclonal antibodies of various portions of a major antigenic site of human growth hormone. *Molec. Immunol.* **19,** 865.

RETEGUI L.A. & PALADINI A.C. (1986) Heteroclitic behaviour of some monoclonal antibodies against bovine growth hormone. *Molec. Immunol.* **23,** 119.

ROTH J. (1975) Methods for assessing immunologic and biological properties of iodinated peptide hormones. In: *Methods in Enzymology* (eds S. O. Colowick and N. O. Kaplan), vol. 37, part B, *Peptide Hormones*, pp. 223. Academic Press, NY.

TAINER J.A., GETZOFF E.D., ALEXANDER H., HOUGHTEN R.A., OLSON A.J. & LERNER R.A. (1984) The reactivity of anti-peptide antibodies is a function of the atomic mobility of sites in a protein. *Nature (Lond.)*, **312,** 127.

VITA N., ETCHEVERRIGARAY M., LÓPEZ BISCAYART P. & RETEGUI L.A. (1986) Relative distribution of various antigenic determinants on the human growth hormone surface. *Molec. Immunol.* **23,** 619.