Complexes of Chromium with β -Glycerophosphate: Their Nature and Properties

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By ion exchange analysis, dialysis and electrophoresis of double labelled (^{32}P and ^{51}Cr) chromic β -glycerophosphate complexes, the stability of the bond β -glycerophosphate-chromium under different conditions of olation and oxolation has been studied. The glycerophosphate can form complexes with 1 or 2 moles per chromium atom. Below pH 6.0 the complexes present cationic characteristics, while between pH 6.0 and pH 8.0 they behave as anions. The oxolation provokes a release of β -glycerophosphate from the complex. The physicochemical characteristics of these complexes are reflected in their behaviour "in vivo" when injected into mice.

In the search for a radioactive compound which, on a metabolic basis, will produce an accumulation of radioactivity "in situ" allowing the scintigraphic scanning or localization of a tumor, the ^{51}Cr - β -glycerophosphate complex has been developed. Previous experimental work¹⁻⁵ has indicated that this compound possesses good properties for a tumor localizing agent.

Considering that a thorough study of the chemical and physicochemical processes involved in preparing this complex would provide a better understanding of its biological behaviour, this experimental study was undertaken.

Material and Methods

a) Preparation of uncomplexed chromic β -glycerophosphate

Stoichiometric quantities (i. e. 0.3 mmol in 5 ml volume) of sodium β -glycerophosphate and chromic chloride were mixed at room temperature and immediately a bulky metallic silver gray precipitate of chromic β -glycerophosphate (**1**) was formed. This precipitate was washed four times with ice cold distilled water.

b) Preparation of complexed chromic β -glycerophosphate

An aqueous suspension of (**1**) was heated in a boiling water bath. The precipitate readily dissolved giving a deep emerald green solution and the pH decreased from 5.0—5.2 to 2.0—2.2. After 10 minutes of

heating the solution was cooled to 5—10 °C and its pH readjusted with 1 M NaHCO_3 . A perfectly clear solution (**2**) was obtained after neutralization, even at pH 8.2. Uncomplexed chromium precipitates at about pH 4.5—5.0.

c) Olated and Oxolated complexes

If the neutralization of (**2**) is carried out at different pH values, a different degree of cationic aggregation is provoked. Also, if this neutralization is accompanied by heating, a higher degree of polymerization (oxolation) is reached.

In all these preparation, the incorporation of ^{51}Cr as CrCl_3 has permitted the radioactive tagging of the complex.

In order to study the stability of the β -glycerophosphate-chromium bond, similar complexes were prepared using ^{32}P - β -glycerophosphate.

d) Ion exchange analysis

One tenth ml samples of each preparation were passed through small columns (7 cm $\times$ 0.7 cm) of anion exchange resin (Dowex 1-X8, chloride form) and cation exchange resin (Dowex 50-X, sodium form). The radioactivity in the eluate as well as that retained on the resins was measured.

e) Dialysis experiments

Two tenths ml samples of the complex were dialyzed for 48 hours against distilled water (with a least 8 changes of the dialysis liquid). The radioactivity on the dialysis bag, as well as an aliquot of the dialysate were counted.

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¹ L. J. ANGHILERI, *Oncology* **21**, 275 [1967].

² L. J. ANGHILERI, *Naturwissenschaften*, Heft 3, S. 136 [1968].

³ L. J. ANGHILERI, *Nuclear Med. [Stuttgart]* **VII**, 266 [1968].

⁴ L. J. ANGHILERI, *Z. Krebsforsch.* **72**, 350 [1969].

⁵ L. J. ANGHILERI, R. C. REBA, and H. N. WAGNER, *Invest. Radiol.* **4**, 91 [1969].

f) *Electrophoretic experiments*

Twenty to fifty μ l samples were analyzed by 1 hour electrophoresis on Whatman 3MM paper, using 0.02 M NaHCO_3 as a buffer and a voltage gradient of 16–18 V/cm. The radioactivity distribution pattern was determined by cutting 1 cm pieces of the electrophoretic strip and counting for ^{51}Cr in a well counter and for ^{32}P in a liquid scintillation counter ⁶.

g) *Assay of the conversion of β -glycerophosphoric acid into the α -form*

The possible transposition of the β -isomer into the α -isomer was assayed according to the method of FLEURY ⁷ based on the action of HIO_4 on the polyhydroxyl compounds.

h) *Study of the animal distribution*

Groups of mice (20–25 gm body weight) were injected intravenously with 0.1 ml of aliquots of labelled complex. The animals were sacrificed at different intervals and the radioactivity of various organs and tissues counted in a well counter. Of whole body counting animals in one group were counted in a large well counter at intervals for 15 days.

Results and Discussion

The solubility of the uncomplexed chromic β -glycerophosphate in 100 ml of water was determined as: 0.210 gm at 4 °C and 0.335 gm at 25 °C. The pH of this solution was 5.0–5.2. After complexing the chromium by boiling, its solubility at 4 °C increases to 10.050 gm and at 25 °C to 13.550 gm, while the pH falls to 2.0–2.2. The influence of the heating in the complex formation can be interpreted as follows:

This process known as anion penetration ⁸ is similar to the tetra-aquo chromic chloride ion formation after heating of a chromic hexahydrate chloride solution ⁹.

Both forms (1) and (2) are insoluble in ethanol. The dehydration of the form (2) with either ethanol or acetone produces a light green powder. This dissolves slowly in water after a hydration characterized by swelling to a deep green gel-like material.

Electrophoretically, form (2), shows a higher mobility toward the anode. Neutrabilized to pH 5.0, it migrates approximately 5–6 cm, while the form (1) moves only 1–2 cm after a 1 hour run using 0.02 M NaHCO_3 as buffer and a voltage gradient of 16 V/cm.

Olation is the process of formation of polynuclear species in which the metal atoms are bound by diol (dihydroxo) bridges. By heating, prolonged aging and/or high pH these polynuclear species can be modified by the elimination of a molecule of water between two adjacent hydroxo bridges with the consequently formation of an Me–O–Me bond. This process is called oxolation ¹⁰. In this case both processes can be described as follows:

Olation

Oxolation

The increase of acidity (release of protons) observed during the preparation of the form (2) is undoubtedly an indication that these processes (particularly olation) are involved in the complex formation.

⁶ T. CLAUSEN, *Analytic. Biochem.* **22**, 770 [1968].

⁷ P. FLEURY, *J. Pharmac. Chim.* **18**, 470 [1933].

* $\beta\text{GPA} = \beta$ -Glycerophosphate.

⁸ F. G. R. GIMBLETT, in: *Inorganic Polymer Chemistry*, p. 104, Butterworths and Co. (Publishers), London 1963.

⁹ F. EPHRAIM, in: *Inorganic Chemistry*, 6th English Ed., p. 314, Oliver and Boyd, London 1954.

¹⁰ F. G. R. GIMBLETT, in: *Inorganic Polymer Chemistry*, p. 77, Butterworths and Co., London 1963.

By submitting the form (2) of double labeled (^{32}P and ^{51}Cr) chromic complex to various degrees of cationic aggregation (increasing of pH with and without heating) the stability of the bond β -glycerophosphate-chromium was studied. Ionic exchange analysis, dialysis and electrophoresis were used to determine to what extent the ^{32}P remains bound to

Fig. 1. Ion exchange analysis and dialysis values of $^{32}\text{P}^{51}\text{Cr}$ -chromic β -glycerophosphate complexes prepared at different pH's.

Fig. 2. Ion exchange analysis and dialysis values of $^{32}\text{P}^{51}\text{Cr}$ -chromic β -glycerophosphate complexes prepared at different pH's and heated to provoke cation aggregation.

the ^{51}Cr under these different conditions. Figs. 1 and 2 show the results of ion exchange analysis and dialysis for complexes prepared at different pH values, without (Fig. 1) and with (Fig. 2) heating to provoke cationic aggregation. These results indicate that the non-dialyzable amount of ^{32}P increases sharply between pH 5.0 and pH 6.0 while between pH 6.0 and pH 8.0 this increase is much lower. The heating also provokes an increase of non-dialyzable ^{32}P between pH 4.0 and pH 6.0, but a significant decrease at higher pH's. On the other hand, the chromium behaviour is similar, but with the characteristic that above pH 6.0 the non-dialyzable fraction increases considerably. If oxolation was produced by heating, the amount of ^{51}Cr complex reaches a value of almost 100% while ^{32}P complex decreases.

The amount of ^{32}P retained by Dowex 1 (anion exchange resin) increases from 23% to approximately 100% when the pH is raised to 7.0, while the amount retained by Dowex 50 (cation exchange resin) decreases from 70% to 16 per cent. When the complex has been heated, the amount of ^{32}P retained by Dowex 1 increases sharply to almost 100% at pH 6.0, but with Dowex 50, it shows a pattern similar to that of the unheated complex; i. e. a decrease from 70–80% at pH 4.0 to 10–20% at pH 6.0 and practically no significant change at higher pH's.

The behaviour of the ^{51}Cr with the ion exchange analysis is strikingly similar to that of the ^{32}P . The most noticeable difference is that between pH 6.0

Fig. 3. Electrophoretic analysis of chromic β -glycerophosphate complexes prepared at different pH's and with or without provoked cation aggregation.

and pH 8.0, the amount of ^{32}P retained by Dowex 50 is slightly higher than the amount of ^{51}Cr .

The electrophoretic determination of free- β -glycerophosphate (not bound to chromium) shows a progressive increase with increased pH, which is still higher (approximately twice) when the preparations are heated (Fig. 3).

We can summarize the results of dialysis, ion exchange analysis and electrophoresis as follows: Under pH 5.0 the complex behaves as a cation, then there is a transition zone (pH 5.0 to 6.0), and over pH 6.0 the complex behaves as an anion. The β -glycerophosphate molecule seems to be tightly bound to the chromium and it is partially released only when the complex is submitted to a drastic cation aggregation (oxolation) by heating. Consequently with the release of β -glycerophosphate, the insolubilization of the chromium is enhanced.

Similarly, using the electrophoretic technique, preparations made with increasing ratios of Na- β -glycerophosphate to chromium (1 to 6) have shown that only complexes with 1 or 2 molecules of β -glycerophosphate per atom of chromium are formed (Fig. 4).

Fig. 4. Effects of the ratio β -glycerophosphate to chromium in the composition of the chromic β -glycerophosphate complex.

Considering the possibility that the sharp decrease in pH (1.1–1.2) and heating during the complex formation could provoke the conversion of β -glycerophosphate into the α -form^{12,13}, the α -glycerophosphate was measured as described elsewhere⁷. No formation of the α -form was detected, which is an indication that the acidity is not enough to catalyze that transformation.

From the biological point of view there is a well defined pattern closely related to the physicochemical properties of the complexes. As can be seen in Tables I and II the increasing of pH and/or heating raises the amount of radioactivity taken up by the R.E.S. (liver) while diminishing the bone uptake. Both phenomena are caused mainly by the polymerization of the chromic- β -glycerophosphate complex, which by progressive aggregation reaches a colloidal

	Un-complexed	Complexed pH 5.0	Complexed pH 5.0 + heating
Blood	1.34 ± 0.29	0.31 ± 0.08	0.20 ± 0.04
Liver	1.62 ± 0.76	4.38 ± 1.17	23.05 ± 0.94
Kidneys	1.75 ± 0.53	0.27 ± 0.02	0.49 ± 0.04
Lungs	1.39 ± 0.44	0.20 ± 0.12	0.36 ± 0.18
Spleen	1.44 ± 0.51	2.66 ± 1.02	6.12 ± 1.36
Bone	9.73 ± 0.87	2.27 ± 0.92	1.99 ± 0.30

Table I. Radioactivity distribution 24 hours after intravenous injection of different types of ^{51}Cr - β -glycerophosphate (as percentage of injected dose per gram of tissue) in groups of 4 animals.

form (becoming non-dialyzable around 100%) that is retained to a higher extent by the Kupffer cells. On the other hand, this polymerization removes the cationic characteristics of the chromium complex and consequently its ability to exchange with calcium and magnesium of the mineral bone tissue¹¹. As has

	pH 4.8	pH 4.8 w. heating	pH 6.0	pH 6.0 w. heating	pH 8.0	pH 8.0 w. heating
Blood	1.22 ± 0.19	0.56 ± 0.07	1.00 ± 0.22	0.79 ± 0.22	0.42 ± 0.09	0.11 ± 0.05
Tumor	1.21 ± 0.47	0.50 ± 0.12	1.08 ± 0.34	0.65 ± 0.11	1.12 ± 0.66	0.18 ± 0.14
Brain	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.005	0.02 ± 0.005	0.02 ± 0.005	0.34 ± 0.12
Muscle	0.37 ± 0.21	0.19 ± 0.11	0.21 ± 0.07	0.09 ± 0.02	0.19 ± 0.07	0.42 ± 0.16
Bone	18.2 ± 5.55	4.78 ± 0.74	11.9 ± 4.88	2.33 ± 1.08	5.11 ± 0.49	5.61 ± 0.27
Liver	0.72 ± 0.19	0.32 ± 0.01	0.96 ± 0.52	1.14 ± 0.13	39.6 ± 2.91	47.5 ± 4.76

Table II. Radioactivity distribution in different tissues 6 hours after intravenous injection of ^{51}Cr - β -glycerophosphate in mice bearing experimental brain tumors (ependymomas). The values are the percentage of injected dose per gram of tissue in groups of 3 animals.

¹¹ L. J. ANGHILERI, *Experientia* [Basel] **25**, 283 [1969].

¹³ E. CHARGAFF, *J. biol. Chemistry* **144**, 455 [1942].

¹² M. C. BAILLY, *C. R. hebd. Séances Acad. Sci.* **206**, 1902 [1938].

	Ratio 1 : 1 w. heating		Ratio 1 : 2 w. heating	
Blood *	0.11 $\pm$ 0.04	0.15 $\pm$ 0.05	0.03 $\pm$ 0.02	0.05 $\pm$ 0.02
Liver	0.31 $\pm$ 0.01	1.44 $\pm$ 0.15	7.45 $\pm$ 0.47	3.44 $\pm$ 0.05
Kidneys	0.43 $\pm$ 0.03	0.37 $\pm$ 0.04	0.67 $\pm$ 0.08	0.54 $\pm$ 0.04
Lungs	0.03 $\pm$ 0.01	0.07 $\pm$ 0.03	0.10 $\pm$ 0.02	0.08 $\pm$ 0.02
Spleen	0.02 $\pm$ 0.01	0.05 $\pm$ 0.02	0.33 $\pm$ 0.06	0.16 $\pm$ 0.01
Bone *	4.36 $\pm$ 1.24	1.78 $\pm$ 0.38	4.01 $\pm$ 0.78	3.65 $\pm$ 0.18

* Percentage of dose per gram.

Table III. Influence of the ratio of chromium to β -glycerophosphate in the biological behaviour of ^{51}Cr - β -glycerophosphate complex (Groups of 4 animals sacrificed 24 hours after intravenous injection and as percentage of injected dose per organ).

been seen before, the increase of pH and heating lowers the amount of β -glycerophosphate in the complex and the two combined effects consequently reduce its availability for metabolic processes. This situation is reflected in the tumor uptake (Table II). Similarly, the number of molecules of β -glycerophosphate incorporated into the chromium complex seems to influence the biological fate of the chromium (Table III).

This direct relationship between cation aggregation and biological behaviour also is corroborated by the whole body counting (Fig. 5) where a higher polymerization corresponds with a higher radioactivity retention by the whole body.

Fig. 5. Whole body counting at different time intervals after intravenous injection of different ^{51}Cr - β -glycerophosphate preparations.

Inhibitoren der Nucleosidphosphorylierung in vivo

Inhibitors of Nucleoside Phosphorylation in vivo

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Acridinorange und pyronine inhibit the uptake and phosphorylation of nucleosides. The same doses of inhibitor do not yet interfere with RNA synthesis. An inhibition of similar specificity concerning nucleoside uptake is observed by several pyrimido-pyrimidine and pteridine derivatives. This inhibition can be reversed only by such nucleosides which are phosphorylated by the same nucleoside kinase. Several nucleoside analogues were also tested. We conclude that the inhibitors are interfering with the kinase reaction in vivo.

The propagation of fowl plague virus (KP) is inhibited only by high doses of pyronine, yet neither by the pyrimido-pyrimidines nor the pteridines tested.

In letzter Zeit sind eine Reihe von Substanzen untersucht worden, die eine spezifische Hemmung der Aufnahme von Nucleosiden in Gewebekulturzellen zeigen. Diese Wirkung wurde bei Acridin-Deriv-

vaten mit sekundärer oder tertiärer Aminogruppe und Pyronin¹ und bei 2-Mercapto-1-(β -4-pyrid-

¹ C. SCHOLTISSEK u. H. BECHT, Biochim. biophysica Acta [Amsterdam] 123, 585 [1966].